

Bioactive potential of *Allamanda cathartica* derived zinc oxide nanoparticles: antimicrobial, antioxidant, and cytotoxic properties with computational mechanistic insights

Gururaj B. Tennalli

KLE Technological University

Basavaraj S. Hungund

hungundb@gmail.com

KLE Technological University

Savitha S. Desai

KLE Society's P.C. Jabin Science College

Leena V. Hublikar

KLE Technological University

Nisha Navada

KLE Technological University

Research Article

Keywords: *Allamanda cathartica*, Zinc oxide nanoparticles, Green synthesis, Cytotoxicity, Density functional theory (DFT), Molecular docking.

Posted Date: September 30th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11193677/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Bioactive potential of *Allamanda cathartica* derived zinc oxide nanoparticles: antimicrobial, antioxidant, and cytotoxic properties with computational mechanistic insights.

**Gururaj B. Tennalli¹, Basavaraj S. Hungund^{1*}, Savitha S. Desai², Leena V. Hublikar³,
Nisha Navada¹**

¹Bioresource Development Laboratory, Department of Biotechnology, KLE Technological University, Hubballi-580031, Karnataka, India.

²P.G. Department of Studies & Research in Biotechnology, KLE Society's P.C. Jabin Science College, Hubballi-580031, Karnataka, India.

³Department of Chemistry, School of Advanced Sciences, KLE Technological University, Hubballi-580031, Karnataka, India.

***Corresponding Author:** Dr. Basavaraj S. Hungund, Department of Biotechnology, KLE Technological University, Hubballi, Karnataka, 580031, India. E-mail: hungundb@gmail.com; M: +91-9449169349

Highlights

- Plant-mediated synthesis produced phase-pure wurtzite ZnO nanoparticles (20–60 nm).
- Surface phytochemicals promoted Zn^{2+} reduction and nanoparticle stabilization.
- ZnO-NPs exhibited antibacterial, antioxidant and selective anticancer activities.
- DFT revealed favorable frontier orbital characteristics for phytochemical-mediated synthesis.
- Docking analysis demonstrated stable ligand interactions with human 17β -HSD1 (PDB: 1FDW).

Abstract

Green synthesis of metal oxide nanoparticles using medicinal plants provides a sustainable approach for developing bioactive nanomaterials. In this study, zinc oxide nanoparticles (ZnO-NPs) were biosynthesized using *Allamanda cathartica* leaf extract and evaluated through an integrated experimental and computational approach. The synthesized ZnO-NPs were characterized by UV–visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDX). UV–visible analysis showed a characteristic absorption maximum at 374 nm, while FTIR confirmed Zn–O bonding and the presence of surface-associated hydroxyl and organic functionalities. XRD analysis indicated a crystalline hexagonal wurtzite ZnO phase, and TEM revealed predominantly spherical nanoparticles approximately 20–60 nm in size. EDX confirmed Zn and O as the major elemental constituents. The biosynthesized ZnO-NPs exhibited broad-spectrum antibacterial activity against *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. DPPH radical-scavenging activity increased with nanoparticle concentration, with an IC_{50} of $655.5 \mu\text{g mL}^{-1}$. MTT analysis demonstrated concentration-dependent cytotoxicity against A549, MCF-7, and KB-3-1 cancer cells, with IC_{50} values of 243.81, 175.92, and $189.62 \mu\text{g mL}^{-1}$, respectively, whereas L929 fibroblasts showed a higher IC_{50} of $640.16 \mu\text{g mL}^{-1}$. The corresponding selectivity indices were 2.63, 3.64, and 3.38 for A549, MCF-7, and KB-3-1 cells, respectively, indicating preferential in-vitro cytotoxicity toward the tested cancer cells relative to L929 fibroblasts. DFT calculations of selected *A. cathartica* phytoconstituents yielded electronic reactivity descriptors that support their potential involvement in nanoparticle formation. At the same time, Monte Carlo simulations indicated energetically favorable adsorption of phytochemical species on the zinc-containing surface. Molecular docking further predicted favorable interactions with the selected biological target (PDB ID: 1FDW), providing complementary mechanistic insight into the observed bioactivity. Overall, *A. cathartica*-mediated ZnO-NPs exhibited combined antibacterial and differential anticancer activity, supporting their potential as bioactive nanomaterials for further biomedical investigation.

Keywords

Allamanda cathartica, Zinc oxide nanoparticles, Green synthesis, Cytotoxicity, Density functional theory (DFT), Molecular docking.

Abbreviations: ZnO-NP – Zinc Oxide nanoparticles; DPPH – 2,2-diphenyl-1-picrylhydrazyl; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; FTIR – Fourier Transform Infrared Spectroscopy; TEM – Transmission Electron Microscopy; XRD – X-ray Diffraction; AC – *Allamanda cathartica*; DFT – Density Functional Theory; ROS – Reactive Oxygen Species; FMO – Frontier Molecular Orbitals; HOMO – Highest Occupied Molecular Orbital; LUMO – Lowest Unoccupied Molecular Orbital; MC – Monte Carlo simulation; MIC – Minimum Inhibitory Concentration.

Introduction

Medicinal plants serve as vital sources of medicinal agents and continue to play a key role in contemporary drug discovery. Their pharmacological activity is primarily attributed to a diverse array of secondary metabolites, including phenolics, flavonoids, alkaloids, terpenoids, and steroids, which confer a wide spectrum of biological activities, such as antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Atanasov et al. 2021; Newman and Cragg 2020). The increasing demand for safer, cost-effective, and biologically compatible therapeutics has renewed attention in plant-borne bioactive complexes and their applications in advanced biomedical technologies (Salem et al. 2023). *Allamanda cathartica* L. (family Apocynaceae) is a therapeutically important plant traditionally used to treat constipation, inflammation, ascites, and poisoning. The ethno-pharmacological relevance of *A. cathartica* is supported by experimental evidence demonstrating its analgesic, antioxidant, thrombolytic, and cytotoxic activities (Tomar et al. 2024; Petricevich and Abarca-Vargas 2019). Phytochemical investigations have confirmed the presence of bioactive compounds, including phenols, alkaloids, flavonoids, and terpenes, which are likely responsible for its diverse pharmacological properties (Ghosh et al. 2019). These results suggest that *A. cathartica* is a promising natural source for developing bio-functional materials. In recent years, the convergence of natural product chemistry and nanotechnology has emerged as an effective strategy for enhancing biological efficacy and expanding therapeutic applications (Patra et al. 2018; Harvey et al. 2015). In particular, the biosynthesis of metal and metallic oxide nanoparticles using plant-based extracts has attracted significant attention for its environmental sustainability, operational simplicity, cost-effectiveness, and improved biocompatibility compared to conventional chemical and physical synthesis routes (Ahmed et al. 2016). Plant-derived biomolecules behave as both reducing and stabilizing mediators during nanoparticle development, thereby influencing particle morphology, size, surface chemistry, and biological interactions. As a consequence, biosynthesized nanoparticles often exhibit enhanced biological activity and reduced toxicity, making them attractive candidates for pharmaceutical and biomedical applications (Mittal et al. 2013).

Due to their favourable physicochemical properties, including nanoscale size, high surface area-to-volume ratio, chemical stability, and intrinsic biocompatibility, zinc oxide nanoparticles (ZnO-NPs) have been widely studied among metallic oxide nanomaterials (Sirelkhatim et al. 2015). The antimicrobial, antioxidant, and anticancer activities of ZnO-NP are mediated mainly by the generation of ROS, membrane disruption, the release of Zn^{2+} ions,

and the induction of oxidative stress, respectively (Abebe et al. 2020; Pushpalatha et al. 2022). Their properties have enabled applications in wound healing, antimicrobial coatings, drug delivery, biosensing, and cancer therapy. Importantly, ZnO-NP have been shown to exhibit differential cytotoxic effects compared to normal cells, making them attractive therapeutic nanomaterials (Jiang et al. 2018; Siddiqi et al. 2018). However, potential toxicity, bioaccumulation, and adverse environmental effects underscore the need for further research to maximize their safe biomedical applications (Bisht & Rayamajhi 2016; Rajput et al. 2020).

Although there is substantial experimental evidence for ZnO-NP bioactivity, the molecular-level mechanisms underlying their biological effects remain only partially understood. In this context, computational approaches complementary to experimental studies, such as density functional theory (DFT) and molecular docking, have proved useful. Insights from DFT calculations on the electronic structure, surface reactivity, stability, and redox behavior of the phytochemicals involved in nanoparticle synthesis have been closely linked to the generation of ROS and antioxidant activity (He et al. 2020; Ghaffar et al. 2023; Mandal 2023). Molecular docking studies allow prediction of the interactions between nanoparticle-associated phytochemicals and biological macromolecules, thus providing mechanistic insight into antimicrobial and cytotoxic effects via potential protein binding and functional inhibition — noting that docking indicates plausible binding compatibility rather than confirmed biological mechanism, and that target selection should be matched to the specific cancer biology under study. Recent studies have increasingly focused on the biosynthesis of ZnO-NP using plant-based extracts, reporting improved antimicrobial efficacy against a broader range of pathogenic bacteria and fungi (Salem and Fouda 2021; El-Saadony et al. 2024). Beyond antimicrobial activity, plant-derived ZnO-NP have also exhibited considerable antioxidant activity, attributed to free-radical scavenging and redox-modulatory capacities arising from surface-bound phytochemicals and electronic properties (Ghaffar et al. 2023). Moreover, several studies have reported their selective toxicity toward cancer cells, suggesting potential use in anticancer therapy (Jiang et al. 2018).

As research focus shifts towards environmentally friendly nanoparticle synthesis, detailed physicochemical characterization, and elucidation of biological activity mechanisms, the present study reports the biosynthesis of ZnO nanoparticles using leaf extract of *Allamanda cathartica* as a natural reducing and stabilizing agent (Sharma et al. 2024). The biosynthesized ZnO-NPs underwent physicochemical characterization and were evaluated for their antimicrobial, antioxidant, and cytotoxic activities against selected microbial strains and cancer

cell lines, the latter benchmarked against the reference chemotherapeutic drug cisplatin. DFT calculations and molecular docking analyses were used to supplement the experimental work, providing molecular-level insights into the electron-donating capacity of the phytoconstituents and their potential interactions with a biologically relevant target protein (Akhter et al. 2024). To the best of our knowledge, no prior study has investigated the synthesis of ZnO-NPs using *A. cathartica* leaf extract or integrated computational DFT and docking analyses to elucidate the mechanistic basis of their bioactivity. This integrated experimental-computational approach is intended to establish the ZnO-NPs synthesized using *A. cathartica* as potential bioactive nanomaterials for therapeutic and biomedical applications. This study reports, for the first time, the phytosynthesis of zinc oxide nanoparticles (ZnO-NPs) using *Allamanda cathartica* leaf extract as a dual reducing and stabilizing agent, integrating comprehensive biological evaluation with multi-level computational validation.

Experimental

Materials

Preparation of *Allamanda cathartica* leaf extract

Allamanda cathartica (fresh leaves) were collected and then washed copiously with distilled water to remove surface dust and impurities. The leaves were air-dried at room temperature and then oven-dried at 55°C to constant weight to ensure complete moisture removal. The dried plant material was ground in a mechanical grinder and sieved to obtain a uniform fine powder. Aqueous extraction was performed by mixing the leaf powder with distilled water at a 1:40 (w/v) ratio in sterile conical flasks. The mixture was macerated for 48 h at room temperature. The extracted suspension was then ultrasonicated (two 10 min cycles) to optimize extraction efficiency, then shaken on a rotary shaker at 120 rpm for 24 h. The extract was filtered through Whatman No. 1 filter paper to remove plant debris. The clear filtrate was freeze-dried to obtain a concentrated crude extract that was stored at 4°C until further analysis (Chemat et al. 2020).

Qualitative phytochemical screening

Primary qualitative phytochemical screening of the lyophilized aqueous leaf extract of *Allamanda cathartica* was carried out to identify major classes of secondary metabolites. Standard biochemical and phytochemical tests were performed to detect carbohydrates (Molisch's test), reducing sugars (Fehling's test), alkaloids (Mayer's and Dragendorff's tests), flavonoids (Shinoda test), glycosides and cardiac glycosides (Keller-Killiani test), tannins and

phenolic compounds (ferric chloride test), saponins (frothing test), steroids and phytosterols (Salkowski and Liebermann-Burchard tests), quinones, starch (iodine test), and coumarins. All tests were conducted in triplicate under standardized laboratory conditions, and a color change or precipitate formation was considered a positive indication of the respective phytochemical group. Screening procedures followed established standard protocols with minor adaptations (Wadood et al. 2013).

Biosynthesis of zinc oxide nanoparticles (ZnO-NP)

Zinc oxide nanoparticles were synthesized using a phyto-mediated precipitation method employing the aqueous leaf extract of *Allamanda cathartica* as a phyto-reducing and stabilizing agent. Briefly, 0.02 M zinc acetate dihydrate [$\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$] was dissolved in 50 mL of deionized water under constant magnetic stirring for 10 min to obtain a clear homogeneous solution. One millilitres of the aqueous leaf extract were then added while stirring continuously. Zinc hydroxide intermediates were formed by dropwise addition of 2 M NaOH to raise the pH, and these intermediates were aged to yield ZnO nanoparticles. The reaction mixture was left undisturbed for 2 h at ambient temperature to ensure complete precipitation. The resulting white precipitate was recovered by centrifugation at 15,000 rpm for 15 min, washed repeatedly with absolute ethanol to remove residual phytoconstituents and unreacted precursors, and then air-dried in a hot-air oven at 60°C overnight. The dried ZnO-NP powder was finely ground and stored in airtight containers under desiccated conditions until further analysis (El-Saadony et al. 2024).

Characterization of ZnO-NP

The optical characteristics of the biosynthesized ZnO-NP were analyzed using UV–Visible spectroscopy in the 200–800 nm wavelength range to detect the characteristic absorption peak and estimate the optical band gap energy. Fourier-transform infrared (FTIR) spectroscopy was used to identify functional groups associated with phytochemical capping and surface interactions. FTIR spectra were recorded using a Nicolet FTIR-6700 spectrometer (Thermo Scientific, USA) at a resolution of 4 cm^{-1} using the KBr pellet technique over the 400–4000 cm^{-1} spectral range. The crystalline structure and phase purity of ZnO-NPs were studied by X-ray diffraction (XRD) using a Bruker D8 Advance X-ray powder diffractometer with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) and a nickel filter over a 2θ range of 10°–90° at a scanning rate of 5° min^{-1} . The average crystallite size was calculated using the Debye–Scherrer equation. Morphological features and particle size distribution were investigated by transmission

electron microscopy (TEM) using a JEOL JEM-2100 instrument at an accelerating voltage of 200 kV. Samples were prepared by drop-casting a dilute nanoparticle suspension onto carbon-coated copper grids, followed by solvent evaporation. Elemental composition was determined by energy-dispersive X-ray spectroscopy (EDX) coupled to a Bruker system (Germany), confirming the presence of zinc and oxygen as the principal constituents (El-Saadony et al. 2024).

Antibacterial activity of ZnO-NPs

The antibacterial activity of the biosynthesized ZnO-NPs was assessed using the agar well diffusion assay against Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria. Fresh overnight cultures were standardized to approximately 0.5 McFarland turbidity ($\approx 10^8$ CFU mL⁻¹). Nutrient agar plates were inoculated by swabbing, and 6 mm diameter wells were created using a sterile corn borer. ZnO-NP suspensions at concentrations of 1250, 2500, 5000, and 10,000 $\mu\text{g mL}^{-1}$ were added to the wells. Sterile distilled water served as the negative control and gentamicin (10 $\mu\text{g mL}^{-1}$) as the positive control. Plates were incubated for 24 h at 37°C under aerobic conditions. The diameters of the inhibition zones were measured in millimeters. All experiments were conducted in triplicate, and results are expressed as mean $\pm$ standard deviation, with one-way ANOVA and Tukey's post hoc test applied for intergroup comparisons (Balouiri et al. 2016).

Minimum inhibitory concentrations (MIC) assay

The MIC of ZnO-NPs was determined using the broth microdilution method in sterile 96-well microplates. Overnight bacterial cultures were standardized to approximately 0.5 McFarland standard ($\approx 10^8$ CFU mL⁻¹). Twenty microliters of standardized bacterial suspension were added to each well containing 100 μL of sterile nutrient broth. ZnO-NP suspensions were prepared in sterile distilled water and two-fold serially diluted to obtain concentrations of 10,000, 5000, 2500, and 1250 $\mu\text{g mL}^{-1}$. A 100 μL volume of each nanoparticle dilution was added to the respective wells (total working volume of 220 μL per well). Following 24 h incubation at 37 °C, 20 μL of aqueous *p*-iodonitrotetrazolium chloride (INT; 4% w/v; Sigma-Aldrich) was added to each well, and the wells were incubated for 30–60 min. The MIC was defined as the minimum concentration that fully inhibited observable bacterial growth, evidenced by the absence of red formazan coloration. All experiments were conducted in triplicate (Kowalska-Krochmal & Dudek-Wicher 2021).

Antioxidant activity (DPPH radical scavenging assay)

Antioxidant activity of the synthesized ZnO-NPs was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. A freshly prepared 0.1 mM DPPH solution in methanol was protected from light before use. ZnO-NP suspensions at concentrations of 125, 250, 500, and 1000 $\mu\text{g mL}^{-1}$ (0.5 mL each) were mixed with 3.5 mL of DPPH solution and kept in the dark at 37°C for 30 min. Absorbance was measured at 517 nm using a UV–Visible double-beam spectrophotometer. The percentage DPPH radical scavenging activity was calculated using the formula:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad (\text{I})$$

Where A_{control} represents the absorbance of the DPPH control, and A_{sample} represents the absorbance in the presence of ZnO-NPs. The IC_{50} value was determined from the dose–response curve. All experiments were conducted in triplicate (Gulcin and Alwasel 2023).

Cytotoxic activity (MTT assay)

The in vitro cytotoxic effects of ZnO-NPs were evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay against human lung carcinoma (A549), human breast adenocarcinoma (MCF-7), human epidermoid carcinoma (KB-3-1), and normal mouse fibroblast (L929) cell lines. All cell lines were obtained from the National Center for Cell Science (NCCS), Pune, India, and confirmed to be mycoplasma-free and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin under standard conditions (37°C, 5% CO_2 , humidified atmosphere). Cisplatin (15 $\mu\text{g mL}^{-1}$) was used as a positive control, and L929 cells were exposed for 24 h under identical conditions to serve as a reference for cytotoxicity and selectivity. Cells were seeded at $\sim 1 \times 10^4$ cells/200 μL per well in 96-well microplates and allowed to adhere for 24 h. The medium was replaced with fresh medium containing ZnO-NP concentrations ranging from 50–250 $\mu\text{g mL}^{-1}$. After 24 h of treatment, 100 μL of MTT solution (0.5 mg mL^{-1}) was added, and the mixture was incubated for 3 h. Formazan crystals were dissolved in 100 μL of DMSO, and absorbance was measured at 570 nm (reference: 630 nm). Cell viability was calculated relative to untreated controls, and IC_{50} values were obtained from dose–response curves by nonlinear regression analysis. Each experiment was repeated three times (Ghaffar et al. 2023).

$$\text{Cell Viability (\%)} = (A_{\text{sample}} / A_{\text{control}}) \times 100 \quad (\text{II})$$

$$\text{Growth Inhibition (\%)} = 100 - \text{Cell Viability (\%)} \quad (\text{III})$$

Determination of IC₅₀ values and selectivity index.

The IC₅₀ (concentration required to inhibit 50 % of cell viability) was calculated from dose–response curves generated using GraphPad Prism 9. All experiments were performed in triplicate, and values were expressed as mean ± standard deviation (SD). The selectivity index (SI) was calculated as: $SI = IC_{50, \text{Normal cells}} / IC_{50, \text{Cancer cells}}$, where the IC₅₀ of HEK-293 normal cells was used as the denominator; SI > 3 was considered indicative of selective cytotoxicity towards cancer cells (Hou et al. 2019).

Computational analysis

The mechanistic aspects of ZnO nanoparticle formation and the role of phytochemicals in the reduction and stabilization process were elucidated using Density Functional Theory (DFT) calculations, Monte Carlo (MC) simulations, and Molecular Docking approaches, applied to the isolated phytoconstituents identified by phytochemical screening rather than to the ZnO nanoparticle lattice itself (Chandramohana et al. 2024).

Density Functional Theory (DFT) calculations

DFT calculations were carried out using the DMol³ module of the BIOVIA Materials Studio software (Accelrys, 2017). Geometry optimization of selected phytochemical molecules was performed without symmetry constraints. The double numerical plus polarization (DNP) basis set was applied along with the generalized gradient approximation (GGA) using the Becke–Lee–Yang–Parr (BLYP) exchange–correlation functional. Solvent effects were incorporated using the conductor-like screening model (COSMO) to simulate an aqueous environment. Frontier molecular orbital (FMO) energies (HOMO and LUMO) were calculated to evaluate electron-donating and -accepting tendencies of these phytoconstituents (not of the ZnO nanoparticle itself). The energy gap ($\Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$) was used as a criterion of chemical reactivity and molecular stability of each ligand. Global reactivity descriptors were computed based on Koopmans' theorem. These parameters were used to assess the reduction potential and stabilization role of phytochemicals in ZnO nanoparticle formation (Parr and Yang 1989; Geerlings et al. 2003).

$$\chi = -(E_{\text{HOMO}} + E_{\text{LUMO}}) / 2 \quad (\text{IV})$$

$$\eta = (E_{\text{LUMO}} - E_{\text{HOMO}}) / 2 \quad (\text{V})$$

$$\sigma = 1/\eta \quad (\text{VI})$$

Monte Carlo (MC) simulation

The adsorption behaviour of selected phytochemicals on ZnO (110) surfaces was studied using Monte Carlo simulation in the Adsorption Locator module of Materials Studio. The ZnO (110) surface was selected for its thermodynamic stability and relevance to crystal growth. Surface models were built using periodic boundary conditions, and a vacuum slab was added to prevent interlayer interactions. An explicit aqueous medium was simulated by incorporating water molecules into the adsorption system. Adsorption configurations were optimized using the COMPASS force field, and adsorption energies (E_{ads}) were calculated to quantify the interaction strength between phytochemicals and the ZnO surface (Parr and Yang 1989; Geerlings et al. 2003).

Molecular docking analysis

The three-dimensional crystal structure of human 17β -hydroxysteroid dehydrogenase type 1 mutant (H221Q) complexed with estradiol was obtained from the Protein Data Bank (PDB ID: 1FDW). The protein structure was prepared by removing co-crystallized ligands and water molecules, followed by adding polar hydrogens and assigning Kollman charges. We drew two-dimensional structures of selected phytochemicals in ChemDraw and converted them into optimized three-dimensional conformations, which we energy-minimized before docking. Molecular docking was performed using PyRx software version 0.8, which integrates AutoDock Vina (Trott and Olsan 2010; Dallakyan and Olsan 2015). We recorded binding affinities (kcal mol^{-1}) and identified the most favorable conformations based on the lowest binding energy. Docked complexes were visualized and analyzed using BIOVIA Discovery Studio Visualizer (ver. 21.1.0.20298) to identify intermolecular interactions, including hydrogen bonding, hydrophobic interactions, and π - π stacking (Kitchen et al. 2004; Meng et al. 2011). Redocking was carried out with X: 45.4, Y: 7.04, Z: 42.35, with spacing of 23.6 oA, 21.5 oA, 18.5 oA, with exhaustiveness of 8.

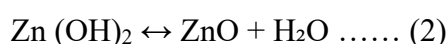
Results and discussion

Qualitative phytochemical analysis

The qualitative phytochemical screening of the *Allamanda cathartica* aqueous leaf extract revealed the presence of a variety of bioactive secondary metabolites. Preliminary biochemical tests confirmed the presence of carbohydrates, reducing sugars, alkaloids, glycosides, flavonoids, cardiac glycosides, phenolic compounds, tannins, saponins, phytosterols, and quinones (Table 1). Among these, alkaloids, flavonoids, phenolic compounds, tannins, saponins, and phytosterols were particularly prominent, suggesting a phytochemical profile rich in redox-active and surface-interacting molecules. The predominance of phenolic and flavonoid compounds is especially relevant in the context of phyto-mediated nanoparticle synthesis. These metabolites are known to exhibit strong electron-donating ability due to hydroxyl and conjugated aromatic systems, enabling them to act as active reducing agents in the conversion of Zn^{2+} ions into ZnO nanostructures (El-Saadony et al. 2024; Nandhini et al. 2024). Furthermore, the presence of saponins and phytosterols may help stabilize ZnO nanoparticles by adsorbing onto their surfaces, thereby preventing aggregation and improving colloidal stability (Mittal and Chisti 2013; Iravani 2011). The detected phytoconstituents therefore provide a biochemical basis for the dual functionality of *A. cathartica* leaf extract as both a stabilizing and reducing agent during ZnO nanoparticle formation. Moreover, many of these metabolites, particularly phenolics and flavonoids, possess well-documented antioxidant and cytotoxic properties that may synergistically contribute to the biological activities of biosynthesized ZnO nanoparticles (El-Saadony et al. 2024; Nandhini et al. 2024).

Biosynthesis of ZnO-NPs

Zinc acetate dihydrate was used as the zinc precursor for the phyto-mediated biosynthesis of ZnO-NPs employing *Allamanda cathartica* (AC) leaf extract. Upon addition of the plant extract to the zinc acetate solution, a gradual color transition from pale yellow to orange-red, and eventually to a deeper reddish hue, was observed, indicative of complexation and redox interactions between Zn^{2+} ions and bioactive phytochemicals that initiate nanoparticle nucleation (Ghaffar et al. 2023; Nandhini et al. 2024). Subsequent adjustment to alkaline conditions (pH $\approx$ 12) produced a milky white colloidal suspension characteristic of ZnO nanoparticle formation. Under strongly basic conditions, Zn^{2+} ions react with hydroxide ions (OH^-) to form zinc hydroxide intermediates that subsequently dehydrate to form ZnO nuclei according to:



Phytochemical analysis of the AC leaf extract confirmed the presence of phenolic compounds, flavonoids, saponins, and tannins with powerful electron-donating and metal-chelating abilities, which participate in stabilization and reduction reactions during nanoparticle formation. The involvement of surface-adsorbed phytochemical biomolecules in the controlled growth and stabilization of ZnO-NP has been widely reported (Ghaffar et al. 2023; El-Saadony et al. 2024; Nandhini et al. 2024). The proposed mechanism for the green synthesis of ZnO-NP using *A. cathartica* leaf extract is illustrated schematically in Fig. 1.

Role of phytochemicals in ZnO-NP formation

The biosynthesis of metal oxide nanoparticles using plant extracts has gained significant attention owing to the eco-friendly nature and the dual role of phytochemicals as reducing and stabilizing agents. The phytochemical screening of the leaf extract of *Allamanda cathartica* revealed abundant flavonoids and phenolic compounds, which are well-documented mediators of metal-ion reduction and nanoparticle stabilization (Ghaffar et al. 2023; Nandhini et al. 2024). These secondary metabolites contain multiple hydroxyl (–OH) and carbonyl (C=O) functional groups with high electron-donating capacity and metal-chelating properties. In the initial step, Zn^{2+} ions are converted to zinc hydroxide intermediates under alkaline conditions, with assistance from electron transfer from the phytochemicals, which then dehydrate to form ZnO nuclei. In the second step, phytochemical molecules adsorb onto the nanoparticle surface, forming a protective organic layer that prevents uncontrolled growth and aggregation, thereby enhancing colloidal stability (El-Saadony et al. 2024; Nandhini et al. 2024). The involvement of these functional groups was further confirmed by FTIR spectroscopy, which revealed shifts in characteristic vibrational bands associated with hydroxyl, carbonyl, and aromatic groups, indicating their coordination with ZnO nanoparticles (Ghaffar et al. 2023; El-Saadony et al. 2024; Nandhini et al. 2024).

Physicochemical characterization of ZnO-NP

Comprehensive physicochemical characterization of the biosynthesized ZnO nanoparticles was performed to assess particle size, morphology, crystallinity, surface functionalization, and elemental composition. These properties play a key role in the catalytic efficiency and biological activity of ZnO nanomaterials. Nanoscale dimensions and surface properties influence surface reactivity, formation of reactive oxygen species (ROS), and interaction with microbial membranes (Ghaffar et al. 2023; El-Saadony et al. 2024). The biomolecule-mediated green synthesis approach provided enhanced dispersion stability and surface functionalization

compared to conventional chemical methods, without the use of toxic reducing agents or synthetic surfactants (Ghaffar et al. 2023; El-Saadony et al. 2024; Nandhini et al. 2024).

UV–Visible spectroscopic analysis

The optical properties and formation of ZnO nanoparticles were investigated using UV–Visible spectroscopy. The absorption spectrum showed a characteristic absorption peak at approximately 374 nm (Fig. 2), coinciding with the native band gap of ZnO due to an electron transition from the valence band to the conduction band. The sharp absorption peak at 374 nm is typical for the wurtzite hexagonal crystal structure of ZnO nanoparticles, confirming the successful synthesis of phase-pure ZnO nanostructures. The relatively narrow absorption band indicates a fine particle size distribution with limited aggregation. These absorption features are commonly observed for plant-mediated ZnO nanoparticles in the range of 360–380 nm, depending on particle size and preparation method (Ghaffar et al. 2023; El-Saadony et al. 2024). The optical band gap energy (E_g) was estimated from a Tauc plot of $(\alpha h\nu)^2$ versus photon energy ($h\nu$) by extrapolating the linear region to $\alpha = 0$. The estimated E_g was 3.32 eV, slightly lower than the reported bulk ZnO band gap of ~ 3.37 eV and comparable to values reported for green-synthesized ZnO nanoparticles in earlier studies (El-Saadony et al. 2024).

Fourier transform infrared (FTIR) spectroscopic analysis.

FTIR spectroscopy was used to identify functional groups associated with the surface of the biosynthesized ZnO nanoparticles and to elucidate the role of phytochemicals in nanoparticle stabilization. The FTIR spectrum (Fig. 3) exhibited a characteristic band at 618 cm^{-1} , which was tentatively attributed to Zn–O lattice vibrations, supporting the formation of ZnO nanoparticles (Ghaffar et al. 2023; Parr & Yang 1989). A broad band around 3363 cm^{-1} indicated the presence of surface hydroxyl groups and adsorbed water molecules, corresponding to O–H stretching vibrations. The bands at 2924 and 2843 cm^{-1} were associated with C–H stretching vibrations, while the band at 1742 cm^{-1} could be attributed to C=O stretching vibrations. The band at 1618 cm^{-1} was associated with O–H bending and/or C=C stretching vibrations, whereas the bands at 1377 and 1246 cm^{-1} may be related to O–H bending and C–O stretching vibrations of residual plant-derived phytochemicals. The prominent band at 1047 cm^{-1} may be attributed to C–O or C–O–C stretching vibrations associated with oxygen-containing phytoconstituents, such as phenolic, alcoholic, or ether-containing compounds, retained on the ZnO-NP surface (Ostovar et al. 2023; Venkatesan et al. 2025). The presence of

these plant-derived functional groups suggests their possible involvement in nanoparticle formation, surface capping, and stabilization.

X-ray diffraction (XRD) analysis

The crystallographic structure, phase purity, and crystalline nature of the green-synthesized ZnO nanoparticles were investigated by XRD. The diffraction pattern (Fig. 4) exhibited characteristic reflections at 2θ values of 31.75° , 34.45° , 36.25° , 47.50° , 56.55° , and 63.25° , corresponding to the (100), (002), (101), (102), (110), and (103) crystallographic planes, respectively. These peaks are in good agreement with the hexagonal wurtzite structure of ZnO (JCPDS card number 36-1451), confirming successful formation of crystalline ZnO nanoparticles (El-Saadony et al. 2024). The absence of additional diffraction peaks indicates high phase purity, with no detectable secondary zinc-containing phases or crystalline impurities. The relatively strong reflection at $2\theta \approx 34.5^\circ$ for the (002) plane indicates preferred growth along the c-axis, commonly observed in ZnO nanostructures prepared under mild or biological conditions (Ghaffar et al. 2023). The crystallite size was estimated using the Debye–Scherrer equation, $D = K\lambda / \beta \cos\theta$, where D is the crystallite size, K is the shape factor (0.9), λ is the Cu K α radiation wavelength (0.15406 nm), β is the full width at half maximum (FWHM) of the diffraction peak in radians, and θ is the corresponding Bragg angle. The calculated crystallite sizes for the major diffraction peaks ranged approximately from 20.3 to 27.5 nm. The relatively small crystallite sizes are consistent with the formation of nanoscale crystalline ZnO. The smaller crystallite size estimated from XRD compared with the TEM particle size range (20–60 nm) is attributable to the different physical parameters probed by the two techniques: XRD estimates the size of coherently diffracting crystalline domains, whereas TEM directly visualizes the overall morphological dimensions of nanoparticles. Thus, a single TEM-observed particle may comprise one or more crystalline domains, resulting in a larger apparent particle size than the XRD-derived crystallite size (Monshi et al. 2012).

Transmission electron microscopy (TEM) and Energy-dispersive X-ray (EDX) analysis

TEM images at various magnifications (Fig. 5) revealed predominantly spherical ZnO nanoparticles with limited agglomeration. Particle size analysis showed ZnO nanoparticles in the 20–60 nm range, indicating successful nanoscale synthesis. The relatively narrow size distribution indicates controlled nucleation and growth processes mediated by plant-derived biomolecules under alkaline conditions (Ghaffar et al. 2023). EDX spectroscopy (Fig. 6) showed strong and distinct peaks for Zn and O, confirming ZnO as the major phase with a

composition of approximately 82.29 wt% zinc and 16.50 wt% oxygen. A small carbon (C) signal was observed, attributable to residual phytochemicals that serve as capping agents or to the carbon support used during TEM sample preparation, as is commonly reported for green-synthesized nanomaterials (El-Saadony et al. 2024). The absence of other elemental peaks confirms the high compositional purity of the biosynthesized ZnO nanoparticles.

Dynamic light scattering (DLS) and zeta potential analysis

Dynamic light scattering (DLS) was used to determine the hydrodynamic particle size of the biosynthesized ZnO-NP suspension in an aqueous medium, yielding a mean hydrodynamic diameter of 86.4 nm and a polydispersity index (PDI) of 0.284. The larger hydrodynamic size observed by TEM, relative to the 20–60 nm particle size, may be attributed to the hydration/solvation shell and a surface-associated phytochemical layer surrounding the nanoparticles, as well as possible particle association in the aqueous dispersion (Kanalli et al. 2025). The ZnO-NP suspension exhibited a zeta potential of -27.6 mV, indicating a moderately stable colloidal dispersion. The negative surface charge may arise from ionizable oxygen-containing functional groups of surface-associated phytoconstituents, which can contribute to electrostatic repulsion and, together with steric effects of the organic capping layer, enhance the colloidal stability of the nanoparticles (Kumar et al. 2025).

Antibacterial activity of ZnO-NPs

The antibacterial activity of the biosynthesized ZnO-NPs was evaluated against selected Gram-positive and Gram-negative bacteria using the agar well diffusion assay. Clear inhibition zones were observed around the wells containing ZnO-NP suspensions at concentrations of 10,000, 5,000, 2,500, and 1,250 $\mu\text{g/mL}$, as summarized in Table 2, indicating inhibitory effects against all tested bacteria. The diameters of the inhibition zones varied among the tested organisms, reflecting differential susceptibility to the nanoparticles. All measurements were performed in triplicate and expressed as mean $\pm$ standard deviation (SD). Several synergistic mechanisms underlie the antibacterial action of ZnO-NPs, including the generation of reactive oxygen species (ROS), the release of Zn^{2+} ions, and direct contact-mediated disruption of the bacterial cell membrane (El-Saadony et al. 2024; Iravani 2011). The nanoscale size and high surface area-to-volume ratio facilitate enhanced interaction with microbial cell walls, promoting oxidative stress and cellular damage. Differences in inhibition efficiency between Gram-negative and Gram-positive bacteria may be attributed to variations in cell wall architecture, with the outer lipopolysaccharide membrane of Gram-negative bacteria potentially limiting

nanoparticle penetration (El-Saadony et al. 2024). One-way ANOVA revealed statistically significant differences in zone of inhibition across all tested concentrations for each organism ($p < 0.001$), confirming that the antibacterial activity of ZnO-NPs is strongly concentration-dependent. The high F-values observed for *Escherichia coli* ($F(3,8)=513.98$) and *Pseudomonas aeruginosa* ($F(3,8)=366.05$) indicate a steep concentration-response relationship. At the same time, the comparatively lower F-value for *Bacillus cereus* ($F(3,8)=58.42$) indicates its sustained susceptibility even at the lowest tested concentration (1,250 $\mu\text{g/mL}$; 8 mm). Tukey's post hoc test confirmed significant pairwise differences among individual concentration groups, thereby validating the dose-dependent antibacterial efficacy of the biosynthesized ZnO-NPs (Balouiri et al. 2016).

Minimum inhibitory concentration (MIC)

The MIC values determined by the microdilution assay were lower for Gram-positive than for Gram-negative bacteria, indicating greater susceptibility of Gram-positive organisms. The MIC assay was performed using the same concentrations of ZnO-NPs (10,000, 5,000, 2,500, and 1,250 $\mu\text{g/mL}$) used for the agar well diffusion assay. However, MIC and zone-of-inhibition measurements represent distinct endpoints: MIC corresponds to the lowest concentration at which no visible bacterial growth is observed in the microdilution assay, whereas the inhibition zone represents the diameter of growth inhibition produced by diffusion of the nanoparticles through agar. Therefore, the absence or size of an inhibition zone at a particular concentration should not be directly equated with the MIC at that concentration. This difference may be attributed to structural differences in cell envelopes: the thick yet permeable peptidoglycan layer in Gram-positive bacteria allows for greater nanoparticle interaction. In contrast, the outer lipopolysaccharide membrane in Gram-negative bacteria serves as an additional permeability barrier (El-Saadony et al. 2024). The small particle size (20–60 nm) and high surface area-to-volume ratio of the synthesized ZnO-NPs facilitated greater surface contact with bacterial cells, thereby enhancing oxidative stress via ROS production and membrane disruption. Overall, the results indicate that plant-mediated ZnO-NPs possess strong antibacterial activity, with greater efficacy against Gram-positive bacteria. All MIC measurements were conducted in triplicate, and the MIC values were determined independently from the inhibition-zone measurements. The MIC replicate data were re-verified to ensure consistency of the reported values (Balouiri et al. 2016).

Antioxidant activity

The DPPH radical scavenging activity of the biosynthesized ZnO-NPs showed a concentration-dependent increase in inhibition, as presented in Fig. 7. The IC_{50} value was determined as $655.5 \mu\text{g mL}^{-1}$. In contrast, ascorbic acid, used as the reference standard, exhibited an IC_{50} value of $832.9 \mu\text{g mL}^{-1}$ under the same experimental conditions. FTIR analysis confirmed the presence of hydroxyl- and phenolic-containing functional groups on the nanoparticle surface, which can contribute electrons or hydrogen atoms to scavenge DPPH radicals. Additionally, the nanoscale particle size (20–60 nm) and high surface area-to-volume ratio enhance interactions with free radicals, improving scavenging efficiency (El-Saadony et al. 2024; Ghaffar et al. 2023). Although Zn^{2+} ions are known cofactors of antioxidant-related enzymes such as superoxide dismutase (SOD), the DPPH assay primarily reflects non-enzymatic radical scavenging activity; thus, the observed antioxidant behavior is more plausibly associated with surface-mediated redox interactions and the contribution of phytochemical residues adsorbed on the nanoparticle surface (El-Saadony et al. 2024). Collectively, these findings indicate that the green-synthesized ZnO nanoparticles exhibit moderate antioxidant potential arising from synergistic effects between their nanostructured surface and bioactive capping agents.

Cytotoxic studies

A549 lung carcinoma cells

The cytotoxic properties of the biosynthesized ZnO-NPs against the A549 human lung carcinoma cell line were assessed using the MTT assay. As shown in Fig. 8, cell viability decreased with increasing ZnO-NP concentration, demonstrating a concentration-dependent cytotoxic effect. The IC_{50} value for A549 cells was calculated as $243.81 \mu\text{g mL}^{-1}$. The decrease in cell viability may be explained by the cellular mechanisms associated with ZnO nanomaterials, including intracellular ROS generation, mitochondrial dysfunction, disruption of membrane integrity, and interference with metabolic pathways by Zn^{2+} ions (Ghaffar et al. 2023; El-Saadony et al. 2024). The nanoscale size (20–60 nm) and large surface area-to-volume ratio facilitate cellular uptake and interactions with cellular machinery, enhancing oxidative stress and activating apoptotic signaling pathways (El-Saadony et al. 2024). Residual phytochemicals adsorbed on the nanoparticle surface may also contribute to cytotoxic effects via redox-mediated interactions and modulation of intracellular signaling pathways (Ghaffar et al. 2023). Phase-contrast microscopy of A549 cells treated with ZnO-NPs (Fig. 8) showed characteristic morphological changes, including cell shrinkage, rounding, membrane blebbing,

and detachment from the culture surface, consistent with apoptotic processes (El-Saadony et al. 2024; Iravani, 2011).

MCF7 breast carcinoma cells

Concentration-dependent cytotoxic activity was observed in the MCF7 human breast carcinoma cell line following treatment with biosynthesized ZnO-NP. As depicted in Fig. 9, cell viability gradually decreased with increasing nanoparticle concentration, yielding an IC_{50} of $175.92 \mu\text{g mL}^{-1}$, indicating greater sensitivity than in A549 cells under identical experimental conditions. Phase-contrast microscopy (Fig. 9) showed marked changes in treated cells, including cell shrinkage, rounding, detachment, and reduced confluency, whereas untreated controls retained normal morphology. These alterations are typical signs of nanoparticle-induced cytotoxic stress and are related to apoptosis and disruption of cellular integrity (El-Saadony et al. 2024). The enhanced cytotoxic response in MCF7 cells may be associated with differences in cellular capacity for nanoparticle uptake, maintenance of intracellular redox balance, and sensitivity to oxidative stress. Mechanisms of ZnO-NP cytotoxicity include ROS production, mitochondrial dysfunction, and activation of apoptotic signaling pathways (Ghaffar et al. 2023; El-Saadony et al. 2024).

Cytotoxic evaluation across multiple cell lines

Cytotoxicity studies were extended to the L929 normal mouse fibroblast cell line and the KB-3-1 human oral carcinoma cell line to assess the biological profile and selectivity of the ZnO-NP. L929 fibroblasts showed lower sensitivity to ZnO-NP treatment in the tested concentration range, with less pronounced morphological changes; at low concentrations, cell architecture remained relatively normal, while partial detachment was observed only at high concentrations (Fig. 10). This indicates comparatively lower cytotoxicity toward non-cancerous cells, an important attribute for assessing therapeutic relevance (El-Saadony et al. 2024).

The KB-3-1 oral carcinoma cell line exhibited a clear concentration-dependent decrease in cell viability, consistent with the antiproliferative responses observed in MCF7 and A549 cells (Fig. 11). Microscopic examination showed rounded cells and cytoplasmic vacuolization after treatment, signs of stress, and decreased viability. The IC_{50} value analysis revealed a sensitivity pattern of $\text{MCF7} > \text{KB-3-1} > \text{A549} > \text{L929}$, indicating greater cytotoxic sensitivity in the cancer cell lines than in the normal fibroblast cell line, with MCF7 showing the greatest sensitivity. This behaviour is consistent with ROS-induced apoptosis reported for ZnO nanoparticles, which is often amplified in cancer cells due to alterations in redox homeostasis

and mitochondrial dysfunction (Iravani 2011). The nano-scale size (20–60 nm) and high surface reactivity of ZnO-NPs facilitate cellular uptake and intracellular Zn²⁺ release, enhancing apoptotic signaling and oxidative stress within cells (Ghaffar et al. 2023). The proposed mechanism of ZnO-NP-induced cytotoxicity is illustrated schematically in Fig. 12.

Selectivity index and possible basis of differential cytotoxicity

To further evaluate the differential cytotoxic response of cancerous and normal cells to the biosynthesized ZnO-NPs, the selectivity index (SI) was calculated using the IC₅₀ value obtained for L929 normal fibroblast cells as the reference. The SI was calculated as the ratio of the IC₅₀ value for L929 cells to that of each cancer cell line: $SI = IC_{50} (L929) / IC_{50} (\text{cancer cell line})$. An SI > 1 indicates greater cytotoxic sensitivity of the cancer cell line relative to the normal fibroblast cells, whereas an SI < 1 indicates comparatively greater sensitivity of the normal cells. The calculated values are presented in Table 3. L929 fibroblast cells exhibited an IC₅₀ of 640.16 µg mL⁻¹, whereas considerably lower IC₅₀ values were obtained for A549 (243.81 µg mL⁻¹), MCF7 (175.92 µg mL⁻¹), and KB-3-1 (189.62 µg mL⁻¹) cells. Accordingly, SI values of 2.63, 3.64, and 3.38 were obtained for A549, MCF7, and KB-3-1 cells, respectively. The SI values, therefore, followed the order MCF-7 > KB-3-1 > A549, consistent with the relative IC₅₀ values observed in the MTT assay.

The SI values greater than 2 observed for all three cancer cell lines indicate preferential cytotoxicity of the ZnO-NPs toward the tested malignant cells relative to L929 fibroblasts under the experimental conditions employed. The highest SI (3.64) was observed in MCF7 cells, indicating that the concentration required to reduce L929 cell viability by 50% was approximately 3.6-fold higher than in MCF7 cells. Similarly, the ZnO-NPs displayed approximately 3.4-fold and 2.6-fold greater sensitivity toward KB-3-1 and A549 cells, respectively, than toward L929 cells. These results provide additional evidence of the differential cellular response to biosynthesized ZnO-NPs and complement the concentration-dependent cytotoxicity observed in the MTT assay. The observed differential cytotoxicity may be associated with differences in cellular redox status, membrane properties, nanoparticle uptake, and susceptibility to oxidative stress. Cancer cells generally exhibit altered redox homeostasis and elevated basal oxidative stress, which can increase their vulnerability to additional ROS generated following exposure to ZnO-NPs. ZnO nanoparticles have been reported to exert cytotoxic effects through several interconnected processes, including nanoparticle–membrane interactions, intracellular ROS generation, Zn²⁺ release, mitochondrial dysfunction, and subsequent activation of cell-death pathways (Iravani 2011; Chattaraj et al.

2003). The morphological alterations observed in the present study, including cell rounding, shrinkage, membrane blebbing, detachment, and cytoplasmic vacuolization, are consistent with substantial cellular stress and loss of viability.

The physicochemical characteristics of the present ZnO-NPs may also contribute to their biological response. TEM analysis revealed predominantly spherical nanoparticles in the 20–60 nm size range, thereby providing a relatively high surface area for interaction with cellular components. Nanoscale ZnO particles can interact with cell membranes and undergo cellular internalization, while dissolution at the particle–cell interface can contribute to intracellular Zn²⁺ availability. These processes, together with ROS generation, may exacerbate oxidative damage to cellular proteins, lipids, and nucleic acids, ultimately impairing mitochondrial and cellular functions (Chattaraj et al. 2003; Sabry et al. 2024). Nevertheless, the present study did not directly quantify intracellular ROS, Zn²⁺ release, or mitochondrial membrane potential; therefore, these processes should be considered plausible mechanisms rather than experimentally demonstrated pathways.

The phytochemical composition of the *Allamanda cathartica* leaf extract may influence the surface characteristics and biological behavior of the biosynthesized ZnO-NPs. Preliminary phytochemical screening identified several classes of secondary metabolites, particularly phenolic compounds, flavonoids, alkaloids, tannins, saponins, and phytosterols. Many of these phytoconstituents contain oxygen- or nitrogen-bearing functional groups that can interact with metal ions and nanoparticle surfaces, potentially contributing to the reduction, capping, and stabilization of plant-mediated ZnO-NPs (Mutukwa et al. 2024). The FTIR spectrum of the synthesized ZnO-NPs exhibited bands corresponding to hydroxyl and other oxygen-containing organic functionalities, supporting the possible association of plant-derived molecules with the nanoparticle surface. Such surface-associated phytochemicals may influence nanoparticle dispersion, surface reactivity, and interactions with biological interfaces, thereby potentially contributing to the observed biological activities.

Computational analysis

Density functional theory (DFT) analysis

To evaluate the electron-donating and electron-accepting properties of the main phytoconstituents from the leaf extract of *Allamanda cathartica*, frontier molecular orbital (FMO) analysis was performed. The distribution of HOMO and LUMO for ferulic acid,

kaempferol, p-coumaric acid, and quercetin is shown in Fig. 13, and the calculated electronic parameters are summarized in Table 4. The HOMO energies of the selected phytochemicals ranged from -8.740 to -8.682 eV, indicating good electron-donating capacity to Zn^{2+} ions in aqueous medium. Among the four phytoconstituents, quercetin exhibited the most negative HOMO energy (-8.740 eV), confirming its superior electron-donating capacity, which is attributed to the presence of five hydroxyl substituents on its flavonoid backbone that facilitate extensive electron delocalization across the conjugated ring system (Sabry et al. 2024). Their LUMO energies ranged from -6.404 to -6.350 eV, and HOMO–LUMO energy gaps ($\Delta E \approx 2.33$ eV) indicate appreciable reactivity suitable for electron transfer processes. Global reactivity descriptors including ionization potential (I), electron affinity (A), global hardness (η), global softness (σ), electronegativity (χ), chemical potential (μ), electrophilicity index (ω), and nucleophilicity index (ϵ) (Table 4) showed moderate hardness ($\eta \approx 1.16$ – 1.17 eV) and appreciable softness ($\sigma \approx 0.85$ eV $^{-1}$), suggesting high molecular polarizability and reactivity. The relatively high electrophilicity index ($\omega \approx 24$ eV) of all four phytoconstituents indicates strong charge-transfer interactions with metal-ion centers, consistent with DFT studies reporting high ω values for phenolic and flavonoid compounds engaged in metal-oxide surface interactions (Chakraborty and Chattaraj 2021). Quercetin further ranked highest in electronegativity ($\chi = 7.572$ eV) and electrophilicity index ($\omega = 24.544$ eV), collectively identifying it as the most potent Zn^{2+} reducing and ZnO surface-coordinating agent among the phytoconstituents studied, which is in agreement with Monte Carlo adsorption simulation findings presented in Section 3.8.2 (Chakraborty & Chattaraj, 2021; Sabry et al., 2024). These electronic descriptors provide evidence of the electron-donating capacity and chemical reactivity of phenolic and flavonoid molecules, suggesting their potential to interact with Zn^{2+} precursor species during ZnO-NP synthesis (Parr and Yang 1989; Geerlings et al. 2003). As the DFT calculations were performed on isolated phytoconstituent molecules, these findings specifically reflect their intrinsic electronic and electron-donating properties rather than the structural stability of the ZnO nanoparticle lattice.

Monte Carlo (MC) simulation studies

Selected phytochemical constituents were studied using Monte Carlo simulations in aqueous media to investigate their adsorption behaviour on the ZnO (110) surface. Optimized adsorption configurations are shown in Fig. 14, and corresponding energy parameters are summarized in Table 5. The calculated adsorption energies were highly negative (-3.91×10^4 kJ mol $^{-1}$ per simulation supercell), confirming that adsorption of phytochemical molecules onto

the ZnO surface is spontaneous and thermodynamically favourable. The large magnitude of the adsorption energy indicates chemisorptive-type molecule–surface interactions rather than simple physisorption, consistent with Monte Carlo simulation studies reporting strong negative adsorption energies for polyphenolic molecules on metal oxide surfaces (Fahimirad et al. 2022). The optimized configurations showed a semi-flat orientation of the phytochemical molecules relative to the ZnO surface, indicating maximum surface coverage and effective passivation. This semi-flat adsorption geometry maximizes the number of simultaneous contact points between the phenolic hydroxyl groups and electrophilic Zn^{2+} sites on the ZnO (110) surface, consistent with the superior electron-donating properties of quercetin identified in DFT analysis (Fahimirad et al., 2022; Sabry et al. 2024). The calculated deformation and binding energies further validated the stability of these interactions. The differential adsorption energy per molecule of extract phytochemical ($dE_{ad}/dN_I = -1.65 \times 10^4 \text{ kJ mol}^{-1}$) being significantly more negative than that of water ($dE_{ad}/dN_I = -4.527 \times 10^3 \text{ kJ mol}^{-1}$) confirms competitive displacement of solvent molecules by phytochemicals from the ZnO surface, supporting their role as effective capping agents (Fahimirad et al. 2022). This remarkable adsorption property supports the proposed function of plant-derived biomolecules as reducing and capping agents in nanoparticle shaping. These computational results support the molecular-level stability of ZnO nanoparticles synthesized using *A. cathartica* extract, consistent with stabilization mechanisms reported for other plant-based nanoparticle systems (Chattaraj et al. 2003; Ghaffar et al. 2023).

Molecular docking analysis

Since the IC_{50} value was lowest for the MCF-7 breast cancer cell line, docking studies were focused on this cell line. To investigate the interaction of ZnO nanoparticle-associated phytochemical ligands with human 17β -hydroxysteroid dehydrogenase type 1 mutant (PDB ID: 1FDW), a protein implicated in hormone-dependent cancers, molecular docking analysis was performed using PyRx with the AutoDock Vina algorithm. 17β -hydroxysteroid dehydrogenase type 1 mutant catalyzes the conversion of estrone to the biologically active estradiol, a key hormone that drives the proliferation of estrogen receptor-positive MCF-7 breast cancer cells. Inhibition of 17β -HSD1 may reduce local estradiol biosynthesis and suppress hormone-dependent tumor growth, making it a biologically relevant target for evaluating the anticancer potential of the identified phytochemicals (Trott and Olson 2010; Dallakyan and Olson 2015). The selection of 17β -HSD1 as the docking target is further supported by the established literature demonstrating that flavonoids, including quercetin and

kaempferol, interact with estrogen receptor-associated enzymes through hydrogen bonding and hydrophobic contacts within the substrate-binding pocket, consistent with their electron-rich flavonoid backbone identified in DFT analysis (Sabry et al. 2024).

The docking results showed that the ligands favorably docked into the receptor's substrate-binding pocket. Calculated binding affinities ranged between -6.5 and -5.7 kcal mol⁻¹, indicating favorable and stable ligand–protein interactions. Detailed interaction analysis (Fig. 15) revealed hydrogen bonding, hydrophobic interactions, and electrostatic contacts that contribute to the stabilization of the docked complexes. Hydrogen bonds are particularly important in determining binding specificity and stability, thereby influencing overall binding affinity (Kitchen et al. 2004; Meng et al. 2011). These interactions indicate physicochemical contacts between surface-associated molecules on the nanoparticles and biologically relevant proteins involved in cancer progression. Redocking of the co-crystallized ligand estradiol into the estrogen receptor α binding site yielded an RMSD of 2.8 Å between the crystallographic and predicted poses. Visual inspection demonstrated substantial overlap of the steroid core and preservation of the native binding orientation, supporting the validity of the docking protocol. Although cytotoxic activity was observed against A549 and KB-3-1 cell lines, molecular docking was focused on the estrogen receptor α (ER α ; PDB: 1FDW) as the representative target based on the highest cytotoxic sensitivity observed in MCF-7 breast cancer cells (IC₅₀ = 175.92 μ g mL⁻¹). Specific validated targets with co-crystallized ligands relevant to A549 (lung adenocarcinoma) and KB-3-1 (oral epidermoid carcinoma) were not pursued in the present study; such target-specific docking for these cell lines is recommended as a direction for future work. While molecular docking does not directly confirm biological activity, it provides a mechanistic framework for the observed cytotoxic activity. In totality, the integrated computational analysis supports experimental observations by providing a proposed mechanistic framework: DFT calculations confirmed favourable electronic characteristics of *A. cathartica* phytoconstituents for Zn²⁺ reduction and surface adsorption, Monte Carlo simulations confirmed spontaneous phytochemical adsorption onto the ZnO surface, and molecular docking highlighted favorable binding affinities with MCF7 breast cancer-related target proteins.

The computational findings provide complementary support for the proposed contribution of this phytochemical. DFT calculations on selected *A. cathartica* phytoconstituents revealed frontier molecular orbital characteristics consistent with their potential involvement in electron-transfer processes during nanoparticle formation. The

relatively high HOMO energy level and low HOMO–LUMO energy gap observed in the investigated compounds indicate their potential as electron donors and chemically reactive character. These electronic properties may facilitate interactions with zinc-containing species during synthesis and contribute to reduction, nucleation, and stabilization. However, the DFT results should be interpreted as molecular-level evidence for the reactivity of the selected phytoconstituents rather than direct proof of the complete ZnO-NP formation mechanism. The Monte Carlo simulation results further indicated favorable interactions between the selected plant-derived molecular species and the zinc surface. The negative adsorption energy obtained from the simulation supports energetically favorable adsorption of the investigated molecules on the zinc surface. Such adsorption may contribute to the formation of an organic interfacial layer around the developing nanoparticles, thereby providing a plausible molecular basis for the stabilizing or capping role of phytochemicals during green synthesis. The combined DFT and Monte Carlo results, therefore, complement the FTIR findings and provide a reasonable mechanistic framework in which plant-derived molecules participate in nanoparticle formation and surface stabilization.

The biological findings can consequently be considered in the context of this phytochemical-functionalized nanoparticle surface. Surface-associated phytochemicals may alter the interaction of ZnO-NPs with microbial and mammalian cells, while the intrinsic physicochemical properties of ZnO contribute to ROS generation and Zn^{2+} -mediated cellular effects. The antibacterial activity observed against both Gram-positive and Gram-negative bacteria, together with the concentration-dependent cytotoxicity against the cancer cell lines, demonstrates the multifunctional biological activity of the synthesized material. The moderate DPPH radical-scavenging activity ($\text{IC}_{50}=655.5 \mu\text{g mL}^{-1}$) further indicates that the nanoparticles possess measurable radical-scavenging capacity. However, this activity should not be equated directly with the ROS-generating mechanisms responsible for nanoparticle-mediated cytotoxicity, as DPPH scavenging is an *in vitro* chemical assay. The molecular docking analysis provides an additional computational perspective on the potential biological interactions of the system. The predicted favorable interactions with the selected protein target suggest that molecular recognition between the investigated bioactive components and the protein may contribute to the observed biological effects. However, docking results represent predicted binding interactions and should therefore be considered supportive rather than definitive evidence of a specific molecular mechanism. Experimental validation using

biochemical or cellular target-engagement assays would be required to conclusively establish such interactions.

Taken together, the experimental and computational findings support a working model in which phytochemicals present in *A. cathartica* leaf extract participate in the formation and stabilization of ZnO-NPs. At the same time, the resulting nanoscale material exhibits biological activity through a combination of surface-mediated interactions, ROS-associated stress, and Zn²⁺-related effects. The comparatively higher IC₅₀ of L929 cells and SI values of 2.63–3.64 indicate preferential cytotoxicity toward the tested cancer cell lines, with MCF7 cells showing the greatest sensitivity. Although these findings are promising, the observed SI should be regarded as evidence of differential in-vitro cytotoxicity rather than definitive therapeutic selectivity. Further studies using additional normal human cell models, intracellular ROS measurements, apoptosis-specific assays, and mechanistic molecular investigations would be required to establish the anticancer selectivity and biological mechanism of the *A. cathartica*-mediated ZnO-NPs. Collectively, these results reinforce the mechanistic understanding of ZnO nanoparticle synthesis and bioactivity using *A. cathartica* as a plant source, supporting their potential as plant-based nano-biomaterials for biomedical applications.

Conclusions

The present study demonstrates the successful green synthesis of zinc oxide nanoparticles (ZnO-NPs) using *Allamanda cathartica* leaf extract as a plant-derived source of reducing and stabilizing phytochemicals. The combined physicochemical characterization using UV–Visible spectroscopy, FTIR, XRD, TEM, and EDX confirmed the formation of crystalline ZnO with a hexagonal wurtzite structure, predominantly spherical morphology, and nanoscale particle dimensions of approximately 20–60 nm. The FTIR findings, together with the phytochemical profile of the leaf extract, suggest that plant-derived functional groups are involved in nanoparticle formation and surface stabilization. The biosynthesized ZnO-NPs exhibited broad-spectrum antibacterial activity against the tested Gram-positive and Gram-negative bacteria, with MIC values indicating concentration-dependent antimicrobial activity. The nanoparticles also showed moderate DPPH radical-scavenging activity, with an IC₅₀ of 655.5 µg mL⁻¹. Cytotoxicity evaluation revealed concentration-dependent inhibition of cancer-cell viability, with MCF-7 cells exhibiting the greatest sensitivity (IC₅₀ = 175.92 µg mL⁻¹), followed by KB-3-1 (189.62 µg mL⁻¹) and A549 cells (243.81 µg mL⁻¹). In comparison, L929 fibroblasts showed a substantially higher IC₅₀ of 640.16 µg mL⁻¹. The corresponding selectivity indices of

3.64, 3.38, and 2.63 for MCF-7, KB-3-1, and A549 cells, respectively, indicate preferential in-vitro cytotoxicity toward the tested cancer cells relative to the normal fibroblast model. These findings, together with the observed morphological alterations, support the potential of the biosynthesized ZnO-NPs as a bioactive nanomaterial with differential cytotoxic effects.

The computational investigations complemented the experimental findings by providing a mechanistic framework for understanding the possible contribution of plant-derived phytochemicals to nanoparticle formation and biological activity. DFT calculations of selected phytoconstituents provided electronic reactivity descriptors consistent with their potential involvement in electron-transfer processes during ZnO formation. Monte Carlo simulations further indicated energetically favorable interactions between the investigated phytochemical species and the zinc surface, supporting their possible contribution to surface adsorption and nanoparticle stabilization. Molecular docking analysis also predicted favorable interactions between the investigated ligand(s) and the selected biological target, providing a computational basis for the possible involvement of protein–ligand interactions in the observed anticancer response. These computational results should be considered complementary evidence and provide hypotheses for experimentally testable mechanisms rather than direct confirmation of the individual molecular pathways. Overall, the integration of phytochemical-mediated synthesis, physicochemical characterization, antimicrobial and antioxidant evaluation, cytotoxicity and selectivity analysis, together with DFT, Monte Carlo, and molecular docking approaches, provides a comprehensive assessment of *A. cathartica*-mediated ZnO-NPs. The findings indicate that the plant-derived phytochemical environment may contribute to nanoparticle formation, surface stabilization, and biological functionality. The preferential cytotoxicity observed toward the tested cancer cell lines, particularly MCF-7 cells, together with the antibacterial activity, highlights the potential of these ZnO-NPs for further investigation in biomedical and pharmaceutical applications. However, additional studies involving intracellular ROS quantification, Zn²⁺-release analysis, apoptosis-specific assays, mechanistic target validation, and in vivo evaluation are required to establish their therapeutic efficacy and safety. To the best of our knowledge, this study represents an integrated computational–experimental investigation combining DFT-derived reactivity descriptors, Monte Carlo adsorption simulations, and molecular docking with the physicochemical and biological characterization of *A. cathartica*-mediated ZnO-NPs. This multidisciplinary approach provides a mechanistic framework linking phytochemical-assisted

nanoparticle formation and stabilization with their observed antimicrobial and preferential anticancer activities.

Acknowledgments The authors acknowledge KLE Technological University for providing financial support and laboratory facilities.

Author contributions GBT and BSH made substantial contributions to the conception and design, data acquisition, analysis, and interpretation of data. LVH contributed to the materials characterizations and related write-up. BSH revised the manuscript critically for important intellectual content. NN and SSD contributed to the DFT and docking studies.

Declaration of competing interests The authors declare that there is no conflict of interest regarding the publication of this work.

Data Availability Data will be made available on request.

Ethical Approval The human cancer cell lines A549, MCF7, and KB-3-1 used in the present study were obtained from the National Center for Cell Science (NCCS), Pune, India, and were handled in accordance with established cell culture protocols. The cell lines were maintained under recommended culture and cryopreservation conditions, with passage history documented during experimental use. Cell cultures were routinely monitored for morphological consistency and contamination, and mycoplasma testing was performed before experimental use. As the study used established human cancer cell lines obtained from a recognized cell repository and did not involve the collection or use of primary human tissues, identifiable patient samples, or personal data, informed consent from human participants was not applicable. Ethical approval for the use of established cell lines was therefore not required from a human-subjects ethics committee, in accordance with the applicable institutional guidelines and regulations.

References

1. Abebe B, Zereffa EA, Tadesse A, Murthy HCA (2020) A review on enhancing the antibacterial activity of ZnO: mechanisms and microscopic investigation. *Nanoscale Res Lett* 15:190. <https://doi.org/10.1186/s11671-020-03418-6>
2. Ahmed S, Ahmad M, Swami BL, Ikram S (2016) A review on plant extracts mediated synthesis of silver nanoparticles for antimicrobial applications. *J Adv Res* 7:17–28. <https://doi.org/10.1016/j.jare.2015.02.007>
3. Akhter H, Sarker Ritu S, Siddique S, Chowdhury F, Chowdhury RT, Akhter S, Hakim M (2024) In silico molecular docking and ADMET prediction of biogenic zinc oxide nanoparticles: characterization, and *in vitro* antimicrobial and photocatalytic activity. *RSC Adv* 14:36209–36225. <https://doi.org/10.1039/D4RA06890D>
4. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT (2021) Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discov* 20:200–216. <https://doi.org/10.1038/s41573-020-00114-z>
5. Balouiri M, Sadiki M, Ibnsouda SK (2016) Methods for *in vitro* evaluating antimicrobial activity: a review. *J Pharm Anal* 6:71–79. <https://doi.org/10.1016/j.jpha.2015.11.005>
6. Bisht G, Rayamajhi S (2016) ZnO nanoparticles: a promising anticancer agent but with toxicity concerns. *Nanomaterials* 6:141. <https://doi.org/10.3390/nano6080141>

7. Chakraborty D, Chattaraj PK (2021) Conceptual density functional theory-based electronic structure principles. *Chem Sci* 12:6264–6279. <https://doi.org/10.1039/D0SC07017C>
8. Chandramohana S, Francis AP, Pajaniradje S, Rajagopalan R (2024) Green and chemical synthesized zinc oxide nanoparticles: evaluation of their anti-proliferative activity against breast cancer cell line – an *in vitro* and *in silico* approach. *Part Sci Technol* 42:1155–1177. <https://doi.org/10.1080/02726351.2024.2364859>
9. Chattaraj PK, Maiti B, Sarkar U (2003) Philicity: a unified treatment of chemical reactivity and selectivity. *J Phys Chem A* 107:4973–4975. <https://doi.org/10.1021/jp034707u>
10. Chemat F, Abert-Vian M, Fabiano-Tixier AS, et al. (2020) A review of sustainable and intensified techniques for extraction of food and natural products. *Green Chem* 22:2325–2353. <https://doi.org/10.1039/C9GC03878A>
11. Dallakyan S, Olson AJ (2015) Small-molecule library screening by docking with PyRx. *Methods Mol Biol* 1263:243–250. https://doi.org/10.1007/978-1-4939-2269-7_19
12. El-Saadony MT, Fang G, Yan S, Alkafaas SS, El Nasharty MA, Khedr SA, Hussien AM, Ghosh S, Dladla M, Elkafas SS, et al. (2024) Green synthesis of zinc oxide nanoparticles: preparation, characterization, and biomedical applications – a review. *Int J Nanomedicine* 19:12889–12937. <https://doi.org/10.2147/IJN.S487188>
13. Fahimirad B, Eshaghi Malekshah R, Arab Chamjangali M, Khaleghi Abasabadi R, Bromand S (2022) Theoretical and experimental study of the photodegradation of methyl orange in the presence of different morphologies of Au-ZnO using Monte Carlo dynamic simulation. *Environ Sci Pollut Res Int* 29:55131–55146. <https://doi.org/10.1007/s11356-022-19657-2>
14. Geerlings P, De Proft F, Langenaeker W (2003) Conceptual density functional theory. *Chem Rev* 103:1793–1874. <https://doi.org/10.1021/cr990029p>
15. Ghaffar A, Sulaiman R, Ashraf D, Tariq I, et al. (2023) Synthesis, characterization, and biomedical applications of zinc oxide nanoparticles. *BME Horiz* 1(3):89–103. [https://doi.org/10.37155/2972-449X-vol1\(3\)-89](https://doi.org/10.37155/2972-449X-vol1(3)-89)
16. Ghosh C, Hazra L, Nag SK (2019) *Allamanda cathartica* Linn. (Apocynaceae): a mini review. *Int J Herb Med* 7(4):29–33.
17. Gülçin İ, Alwasel SH (2023) DPPH radical scavenging assay. *Processes* 11:2248. <https://doi.org/10.3390/pr11082248>
18. Harvey AL, Edrada-Ebel R, Quinn RJ (2015) The re-emergence of natural products for drug discovery in the genomics era. *Nat Rev Drug Discov* 14:111–129. <https://doi.org/10.1038/nrd4510>
19. He E, Qiu R, Cao X, Song L, Peijnenburg WJGM, Qiu H (2020) Elucidating toxicodynamic differences at the molecular scale between ZnO nanoparticles and ZnCl₂ in *Enchytraeus crypticus* via nontargeted metabolomics. *Environ Sci Technol* 54:3487–3498. <https://doi.org/10.1021/acs.est.0c00663>
20. Hou J, Wang L, Wang C, Zhang S, Liu H, Li S (2019) Selectivity index as a metric for nanoparticle cytotoxicity: a comparative analysis. *Nanomedicine* 14:1003–1015.
21. Iravani S (2011) Green synthesis of metal nanoparticles using plants. *Green Chem* 13:2638–2650. <https://doi.org/10.1039/C1GC15386B>
22. Jiang J, Pi J, Cai J (2018) The advancing of zinc oxide nanoparticles for biomedical applications. *Bioinorg Chem Appl* 2018:1062562. <https://doi.org/10.1155/2018/1062562>
23. Kanalli P, Kundapur RR, Joseph MM (2025) Betel leaf-mediated zinc oxide nanoparticle-coated silk fibres: a sustainable approach for biomedical applications. *RSC Adv* 15:31162–31175. <https://doi.org/10.1039/D5RA03600C>
24. Kitchen DB, Decornez H, Furr JR, Bajorath J (2004) Docking and scoring in virtual screening for drug discovery: methods and applications. *Nat Rev Drug Discov* 3:935–949. <https://doi.org/10.1038/nrd1549>

25. Kowalska-Krochmal B, Dudek-Wicher R (2021) The minimum inhibitory concentration of antibiotics: methods, interpretation, clinical relevance. *Pathogens* 10:165. <https://doi.org/10.3390/pathogens10020165>
26. Kumar A, Kaur A, Singh MV, Vinod, Dhiman S (2025) Rice bagasse extract-based green synthesis of zinc oxide nanoparticles: characterisation, assessment of anti-skin cancer, antibacterial, and antioxidant properties. *Sustain Chem Clim Action* 7:100118. <https://doi.org/10.1016/j.scca.2025.100118>
27. Mandal AK (2023) Nanomaterials as targeted delivery system of therapeutics for inhibition of cancer. *J Drug Deliv Ther* 13(12). <https://doi.org/10.22270/jddt.v13i12.6014>
28. Meng XY, Zhang HX, Mezei M, Cui M (2011) Molecular docking: a powerful approach for structure-based drug discovery. *Curr Comput Aided Drug Des* 7:146–157. <https://doi.org/10.2174/157340911795677602>
29. Mittal AK, Chisti Y, Banerjee UC (2013) Synthesis of metallic nanoparticles using plant extracts. *Biotechnol Adv* 31:346–356. <https://doi.org/10.1016/j.biotechadv.2013.01.003>
30. Monshi A, Foroughi MR, Monshi MR (2012) Modified Scherrer equation to estimate more accurately nanocrystallite size using XRD. *World J Nano Sci Eng* 2:154–160. <https://doi.org/10.4236/wjnse.2012.23020>
31. Mutukwa D, Taziwa RT, Khotseng L (2024) A review of plant-mediated ZnO nanoparticles for photodegradation and antibacterial applications. *Nanomaterials* 14:1182. <https://doi.org/10.3390/nano14141182>
32. Nandhini J, Karthikeyan E, Rajeshkumar S (2024) Green synthesis of zinc oxide nanoparticles: eco-friendly advancements for biomedical marvels. *Resour Chem Mater* 3:294–316. <https://doi.org/10.1016/j.rcm.2024.05.001>
33. Newman DJ, Cragg GM (2020) Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J Nat Prod* 83:770–803. <https://doi.org/10.1021/acs.jnatprod.9b01285>
34. Ostovar N, Mohammadi N, Khodadadeh F (2023) Photocatalytic, antioxidant and antibacterial potential of bio-synthesized ZnO nanoparticles derived from espresso spent coffee grounds: optimization by central composite design. *Inorg Nano-Met Chem* 53:938–949. <https://doi.org/10.1080/24701556.2023.2187419>
35. Parr RG, Yang W (1989) *Density-functional theory of atoms and molecules*. Oxford University Press, New York.
36. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS, Diaz-Torres LA, Grillo R, Swamy MK, Sharma S, Habtemariam S (2018) Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnol* 16:71. <https://doi.org/10.1186/s12951-018-0392-8>
37. Petricevich VL, Abarca-Vargas R (2019) *Allamanda cathartica*: a review of the phytochemistry, pharmacology, toxicology, and biotechnology. *Molecules* 24(7):1238. <https://doi.org/10.3390/molecules24071238>
38. Pushpalatha C, Suresh J, Gayathri VS, Sowmya SV, Augustine D, Alamoudi A, Zidane B, Albar NHM, Patil S (2022) Zinc oxide nanoparticles: a review on its applications in dentistry. *Front Bioeng Biotechnol* 10:917990. <https://doi.org/10.3389/fbioe.2022.917990>
39. Rajput V, Minkina T, Sushkova S, et al. (2020) ZnO and CuO nanoparticles: a threat to soil organisms, plants, and human health? *Environ Geochem Health* 42:147–168. <https://doi.org/10.1007/s10653-019-00320-1>
40. Sabry NM, Badry R, Abdel-Gawad FK, Elhaes H, Ibrahim MA (2024) Electronic structure, global reactivity descriptors and nonlinear optical properties of glycine interacted with ZnO, MgO and CaO for bacterial detection. *Sci Rep* 14:22801. <https://doi.org/10.1038/s41598-024-72846-6>
41. Salem SS, Fouda A (2021) Green synthesis of metallic nanoparticles and their prospective biotechnological applications: an overview. *Biol Trace Elem Res* 199:344–370. <https://doi.org/10.1007/s12011-020-02138-3>
42. Salem SS, Hammad EN, Mohamed AA, El-Dougdoug W (2023) A comprehensive review of nanomaterials: types, synthesis, characterization, and applications. *Biointerface Res Appl Chem* 13:41. <https://doi.org/10.33263/BRIAC131.041>

43. Sharma V, Kumar G, Kumar P, Kumar D, Nemiwal M (2024) Green synthesis of ZnO nanoparticles from *Dysoxylum binectariferum* fruit extract and assessment of antimicrobial potential. *ChemistrySelect* 9. <https://doi.org/10.1002/slct.202402125>
44. Siddiqi KS, ur Rahman A, Tajuddin, Husen A (2018) Properties of zinc oxide nanoparticles and their activity against microbes. *Nanoscale Res Lett* 13:141. <https://doi.org/10.1186/s11671-018-2532-3>
45. Sirelkhatim A, Mahmud S, Seeni A, et al. (2015) Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-Micro Lett* 7:219–242. <https://doi.org/10.1007/s40820-015-0040-x>
46. Tomar R, Das SS, Sahu V, Kapoor N, Chaudhary D, Sahoo J, Rath SK, Kesari KK (2024) *Allamanda cathartica* (Linn.) leaf extract-encapsulated phytoemulsions: formulation, characterization and *in vitro*–*in vivo* biological evaluations. *Sustain Food Technol* 2:1490–1505. <https://doi.org/10.1039/D4FB00077C>
47. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 31:455–461. <https://doi.org/10.1002/jcc.21334>
48. Venkatesan R, Kanagaraj T, Alrashed MM, et al. (2025) Green synthesis of strontium stannate nanorods using extract of *Juniperus communis* L.: structural characterization and evaluation of antibacterial, antifungal, and antioxidant activity. *Sci Rep* 15:32166. <https://doi.org/10.1038/s41598-025-14412-2>
49. Wadood A, Ghufuran M, Jamal SB, Naeem M, Khan A, Ghaffar R (2013) Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochem Anal Biochem* 2:144. <https://doi.org/10.4172/2161-1009.1000144>

Tables

Table 1 Qualitative phytochemical analysis of *Allamanda cathartica* leaf extract.

Sr. No.	Phytochemical tests	Results
1.	Test for Carbohydrates (Molisch's test)	+
2.	Test for Reducing sugars (Fehling's test)	+
3.	Test for Alkaloids	+
4.	Test for Flavonoids	+
5.	Test for Glycosides	-
6.	Test for Cardiac glycosides (Keller-Killiani test)	+
7.	Test for Phenols	+
8.	Test for Saponins	+
9.	Test for Steroids	-
10.	Test for Quinones	+
11.	Test for Tannins	+
12.	Test for Coumarins	-
13.	Test for Anthraquinones	-
14.	Test for Phytosterols	+

Table 2 Antibacterial activity of ZnO-NP against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.

Test organisms	Diameter of zone of inhibition (in mm)				MIC MIC $\mu\text{g/mL}$
	10000 $\mu\text{g/mL}$	5000 $\mu\text{g/mL}$	2500 $\mu\text{g/mL}$	1250 $\mu\text{g/mL}$	
<i>Staphylococcus aureus</i>	14.00 $\pm$ 0.82	12.00 $\pm$ 0.71	5.00 $\pm$ 0.58	0	2500
<i>Bacillus cereus</i>	16.00 $\pm$ 0.94	14.00 $\pm$ 0.82	12.00 $\pm$ 0.71	8.00 $\pm$ 0.58	1250
<i>Pseudomonas aeruginosa</i>	8.00 $\pm$ 0.58	6.00 $\pm$ 0.47	0	0	5000
<i>Escherichia coli</i>	12.00 $\pm$ 0.71	8.00 $\pm$ 0.58	0	0	5000

Table 3 IC₅₀ and selectivity index of ZnO-NP against cancer and normal cell lines

Cell line	Cell type	IC ₅₀ ($\mu\text{g mL}^{-1}$)	SI
A549	Lung carcinoma	243.81	2.63
MCF-7	Breast carcinoma	175.92	3.64
KB-3-1	Oral carcinoma	189.62	3.38
L929	Normal fibroblast	640.16	1.00

Calculation: SI = IC₅₀ (L929)/IC₅₀ (cancer cell line).

An SI >2 is commonly interpreted as indicating preferential/selective cytotoxicity toward cancer cells relative to normal cells. Therefore, your results provide a useful additional dimension beyond simply comparing IC₅₀ values.

Table 4 Frontier molecular orbital energies (HOMO, LUMO) and global reactivity descriptors calculated for selected phytochemical ligands using density functional theory (DFT).

Name of the compound	E (HOMO) (eV)	E(LUMO) (eV)	ΔE (eV)	A(eV)	η (eV)	χ (eV)	σ (eV ⁻¹)	μ (eV)	ω (eV)	ξ (eV ⁻¹)
Ferulic acid	-8.692	-6.357	2.335	6.357	1.167	7.524	0.856	- 7.524	24.254	0.041
Kaempferol	-8.711	-6.37	2.341	6.37	1.170	7.540	0.854	- 7.540	24.295	0.041
p-coumaric acid	-8.682	-6.350	2.332	6.350	1.166	7.516	0.857	- 7.516	24.223	0.041
Quercetin	-8.740	-6.404	2.336	6.404	1.168	7.572	0.856	- 7.572	24.544	0.040

Table 5 Adsorption and energy parameters obtained from Monte Carlo simulation analysis describing interactions between phytochemical ligands and the ZnO (110) surface.

Total energy	Adsorption energy	Rigid adsorption energy	Deformation energy	dE_{ad}/dN_I (extract)	dE_{ad}/dN_I (Water)
6.058	-3.91×10^{-4}	-7.53	-3.91×10^{-4}	-1.65×10^{-4}	-4.527×10^{-3}

Figures

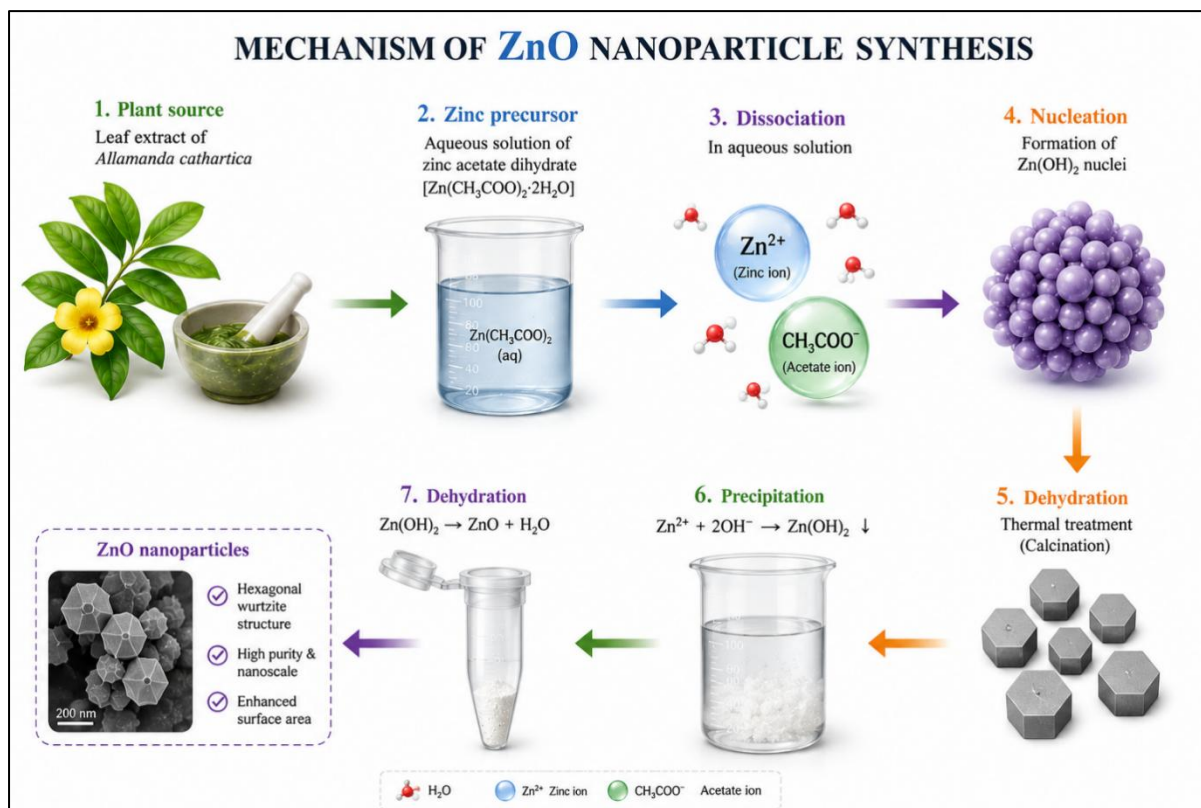


Fig. 1 Proposed mechanism for the green synthesis of ZnO nanoparticles using *Allamanda cathartica* leaf extract, illustrating phytochemical-mediated reduction of Zn^{2+} ions and stabilization of the resulting ZnO nanostructures.

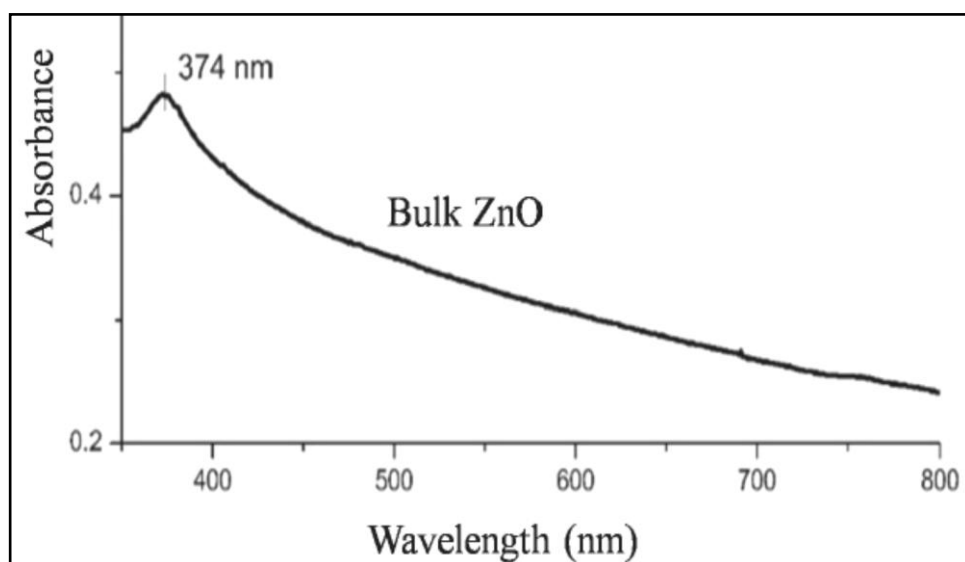


Fig. 2 UV–Visible absorption spectrum of biosynthesized ZnO nanoparticles recorded in the wavelength range of 200–800 nm showing the characteristic absorption peak associated with ZnO band-gap transition.

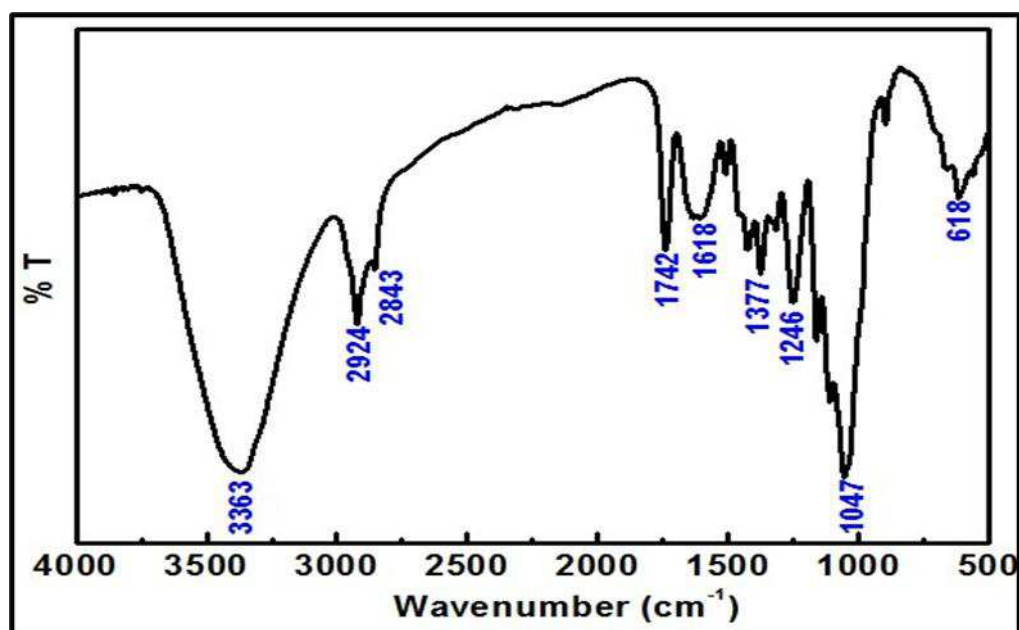


Fig. 3 FTIR spectrum of biosynthesized ZnO nanoparticles indicating Zn–O lattice vibrations and functional groups corresponding to phytochemical capping agents derived from *A. cathartica* extract.

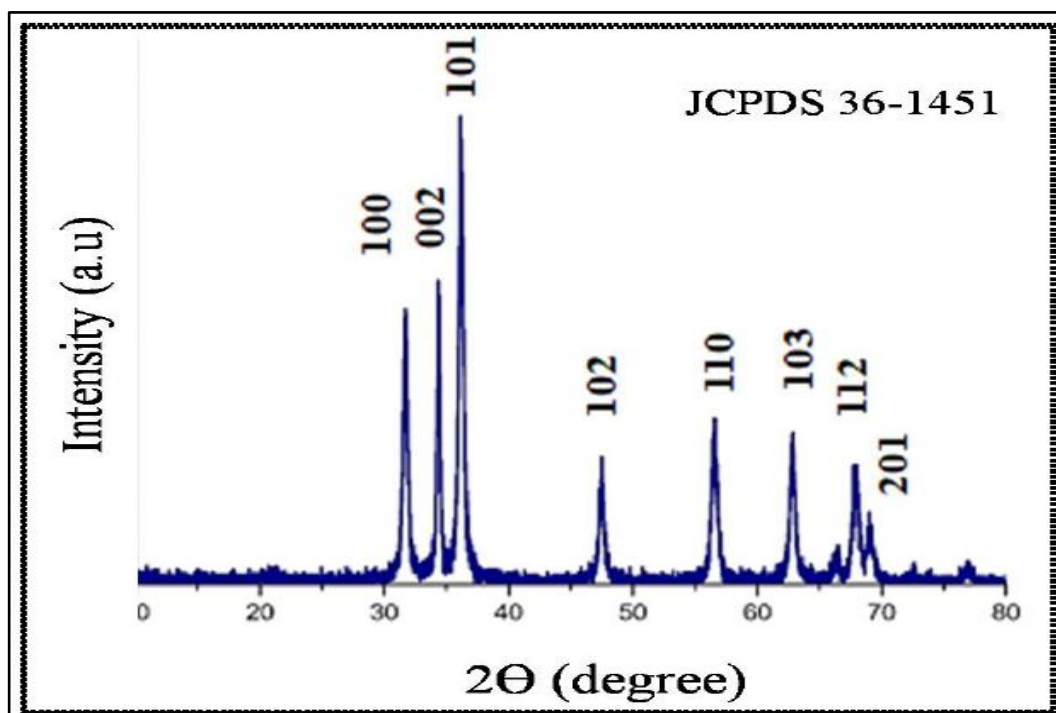


Fig. 4 X-ray diffraction (XRD) pattern of ZnO nanoparticles synthesized using *Allamanda cathartica* leaf extract showing diffraction peaks corresponding to the hexagonal wurtzite crystal structure (JCPDS No. 36-1451).

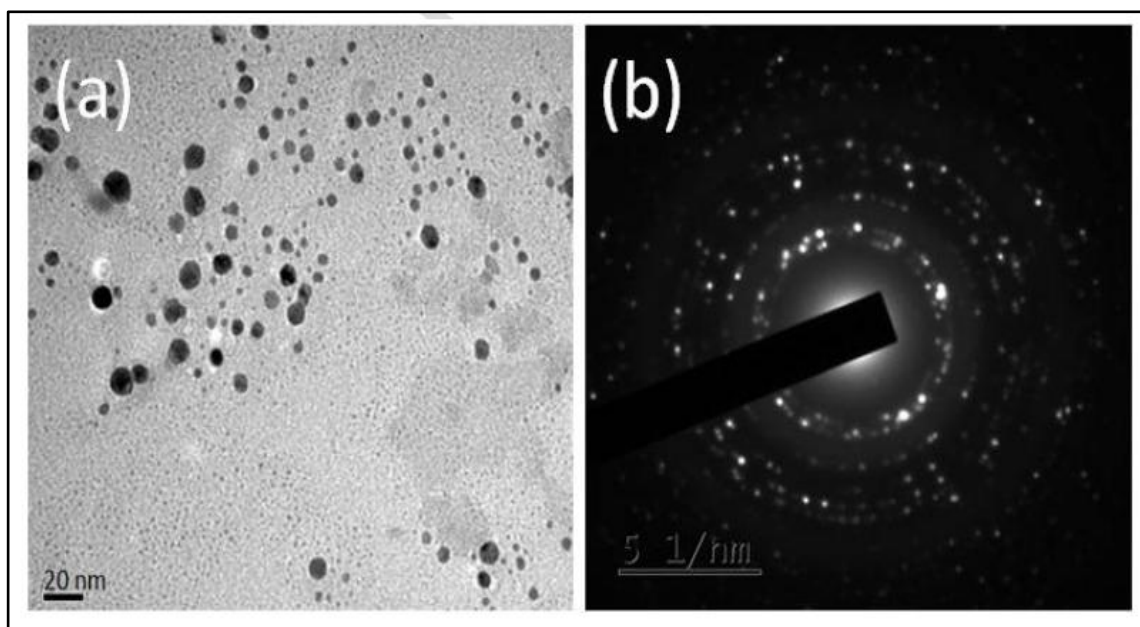


Fig. 5 Transmission electron microscopy (TEM) images of biosynthesized ZnO nanoparticles at different magnifications illustrating particle morphology and size distribution.

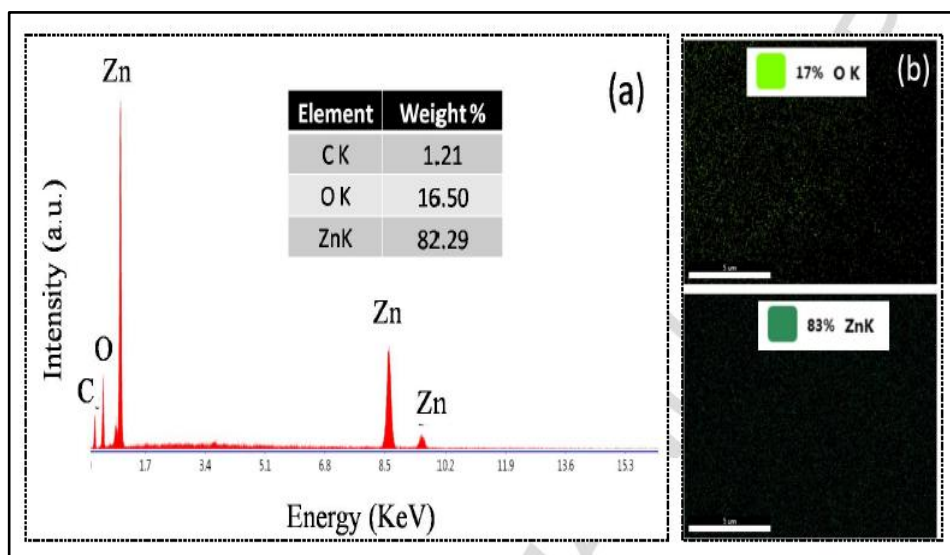


Fig. 6 Energy-dispersive X-ray (EDX) spectrum of ZnO nanoparticles confirming the elemental composition and presence of Zn and O as the principal components.

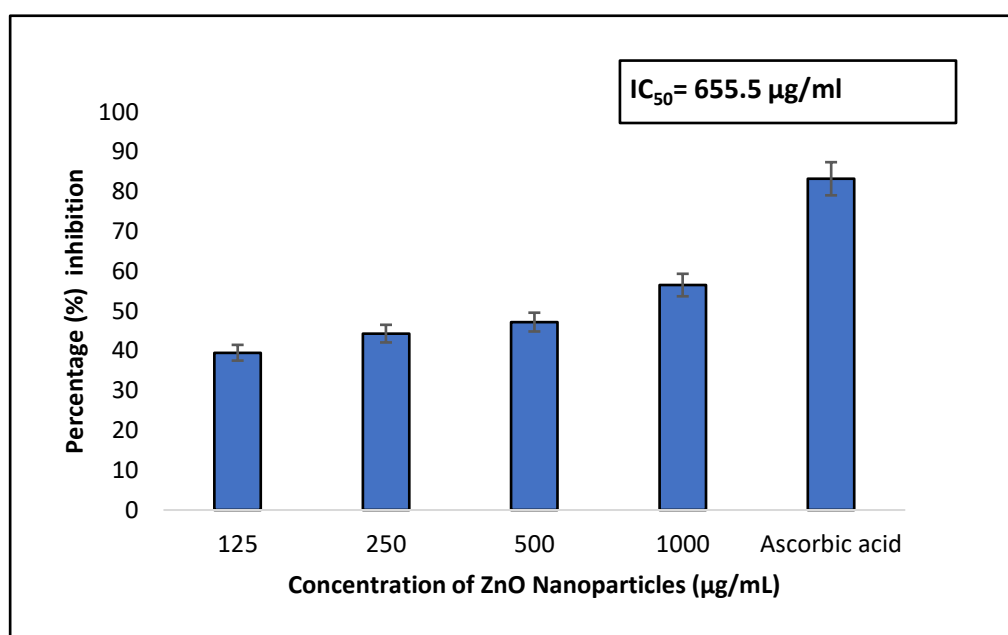


Fig. 7 DPPH radical scavenging activity of biosynthesized ZnO nanoparticles at different concentrations demonstrating concentration-dependent antioxidant activity.

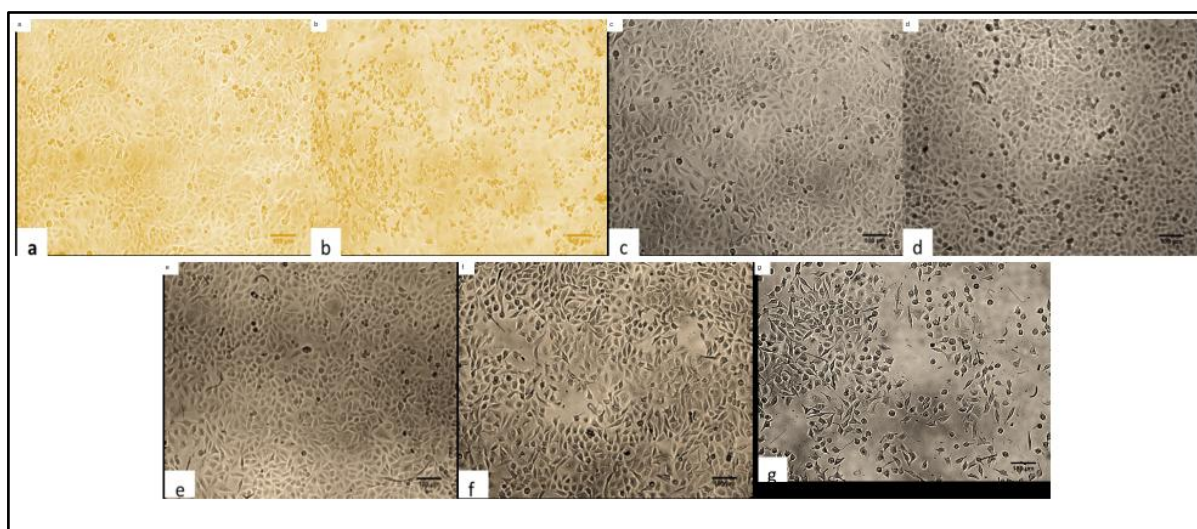
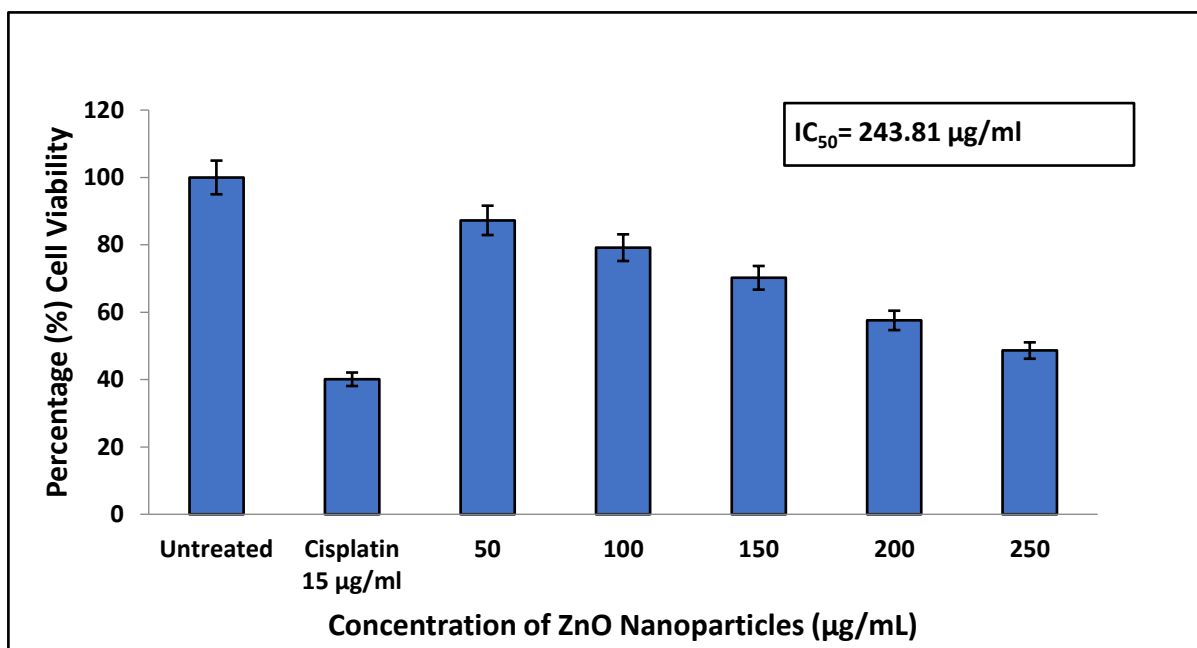


Fig. 8 Cytotoxic effect of A549 lung carcinoma cells by ZnO nanoparticles. MTT assay: cell viability decreased in a dose-dependent manner after 24 h treatment, phase-contrast images: morphological changes after 24 h treatment. (A) Control; (B) Cisplatin-treated; (C–G) ZnO-NP-treated cells at 50, 100, 150, 200, and 250 µg/mL, respectively. With the increase in the concentration of ZnO-NPs, progressive shrinkage, rounding, and detachment of the cells were seen.

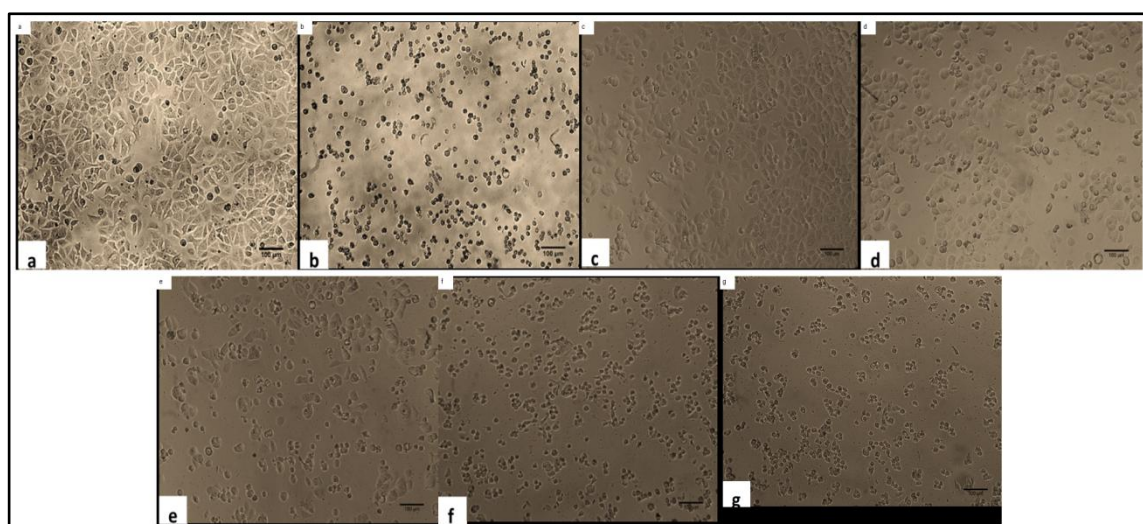
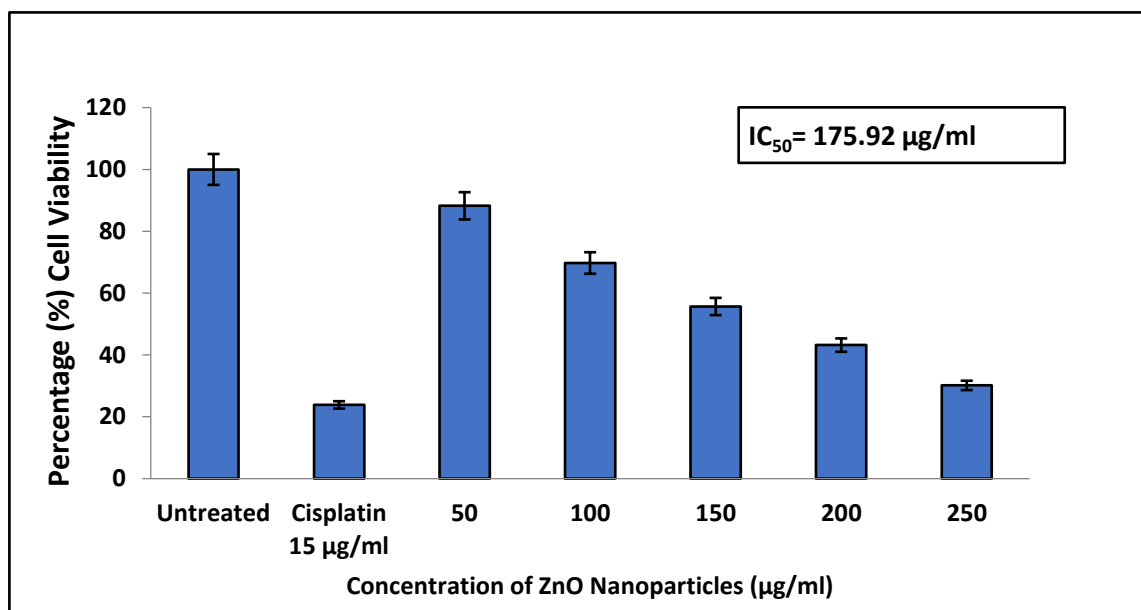


Fig. 9 The cytotoxic and morphological effects of Zinc Oxide Nanoparticles on MCF-7 breast cancer cells. MTT assay indicated the concentration-dependent decrease in cell viability, and phase-contrast images showed the morphological changes after 24 h treatment. (A) Control; (B) Cisplatin-treated; (C–G) ZnO-NP-treated cells at 50, 100, 150, 200, and 250 µg/mL, respectively. As the concentration of ZnO-NPs increased, progressive shrinkage, rounding, and detaching of cells were seen.

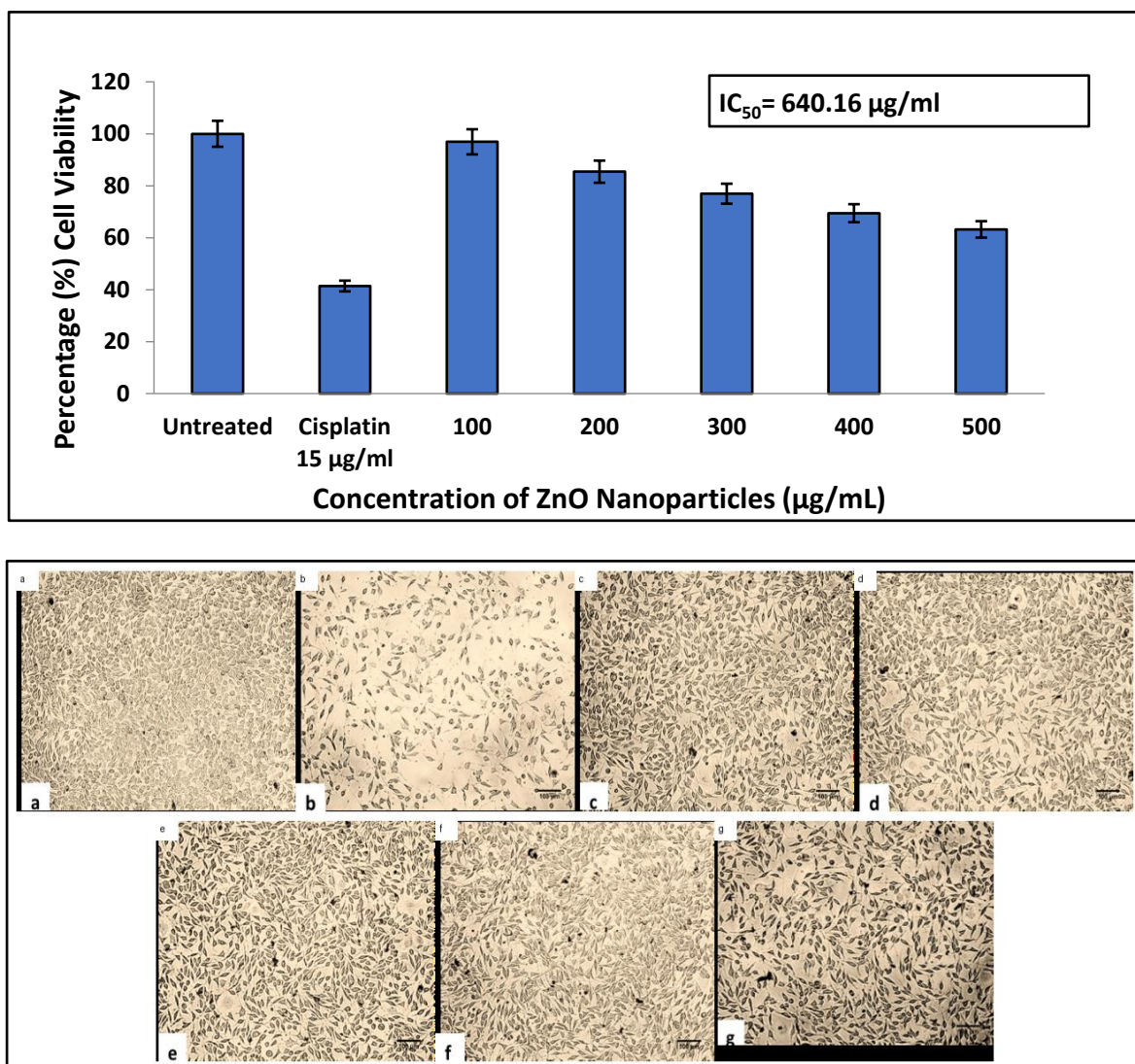


Fig. 10 Effects of ZnO nanoparticles on L929 mouse fibroblast cells. MTT assay showing cell viability following ZnO nanoparticle treatment and phase-contrast images depicting morphological changes after 24 h exposure. (A) Control; (B) Cisplatin-treated; (C–G) ZnO-NP-treated cells at 50, 100, 150, 200, and 250 μg/mL, respectively. Morphological alterations, including cell shrinkage and reduced adhesion, increased with ZnO-NP concentration.

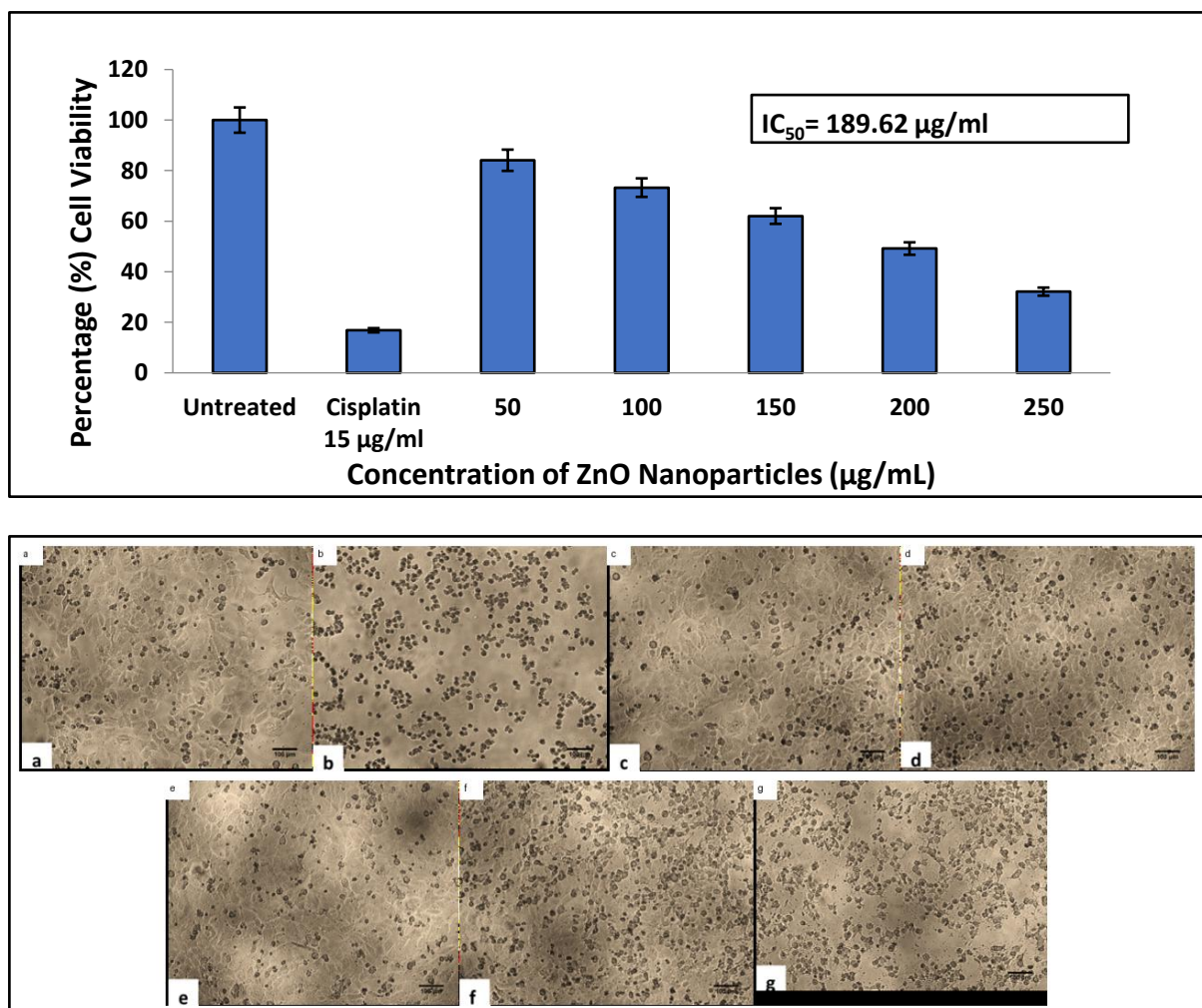


Fig. 11 The cytotoxicity and morphological effects of ZnO nanoparticles on KB-3-1 oral carcinoma cells. MTT cell viability assay (cell viability concentration dependent) and phase-contrast images (morphological changes after 24 h treatment). (A) Control; (B) Cisplatin-treated; (C–G) ZnO-NP-treated cells at 50, 100, 150, 200, and 250 µg/mL, respectively. As the concentration of ZnO-NPs increased, progressive reduction in size, rounding, and loss of adhesion were seen.

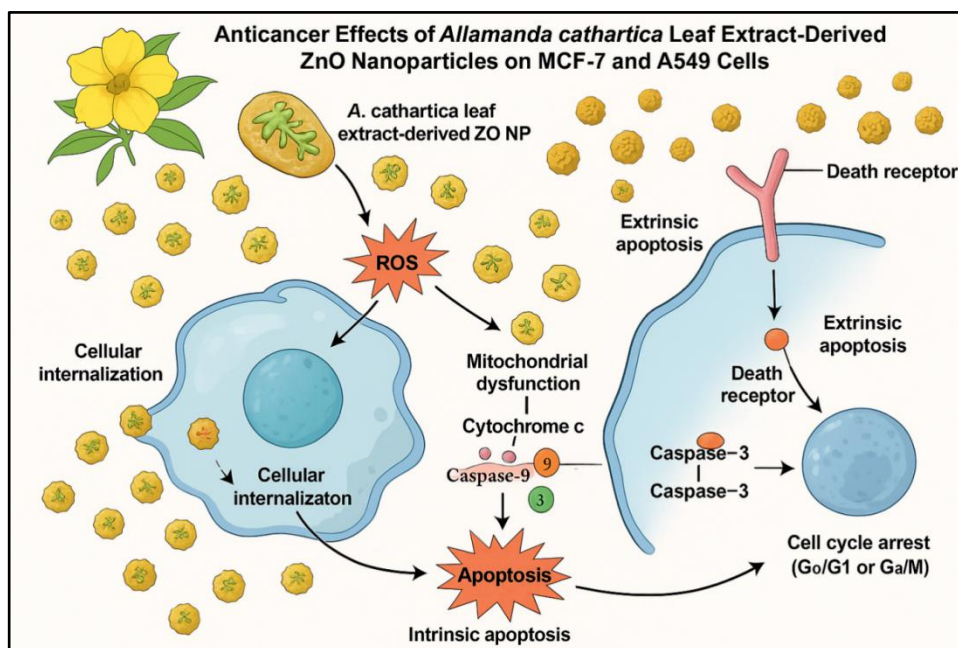


Fig. 12 Proposed schematic mechanism of ZnO nanoparticle-induced cytotoxicity in cancer cells involving ROS generation, mitochondrial dysfunction, Zn²⁺ ion release, and activation of apoptotic pathways.

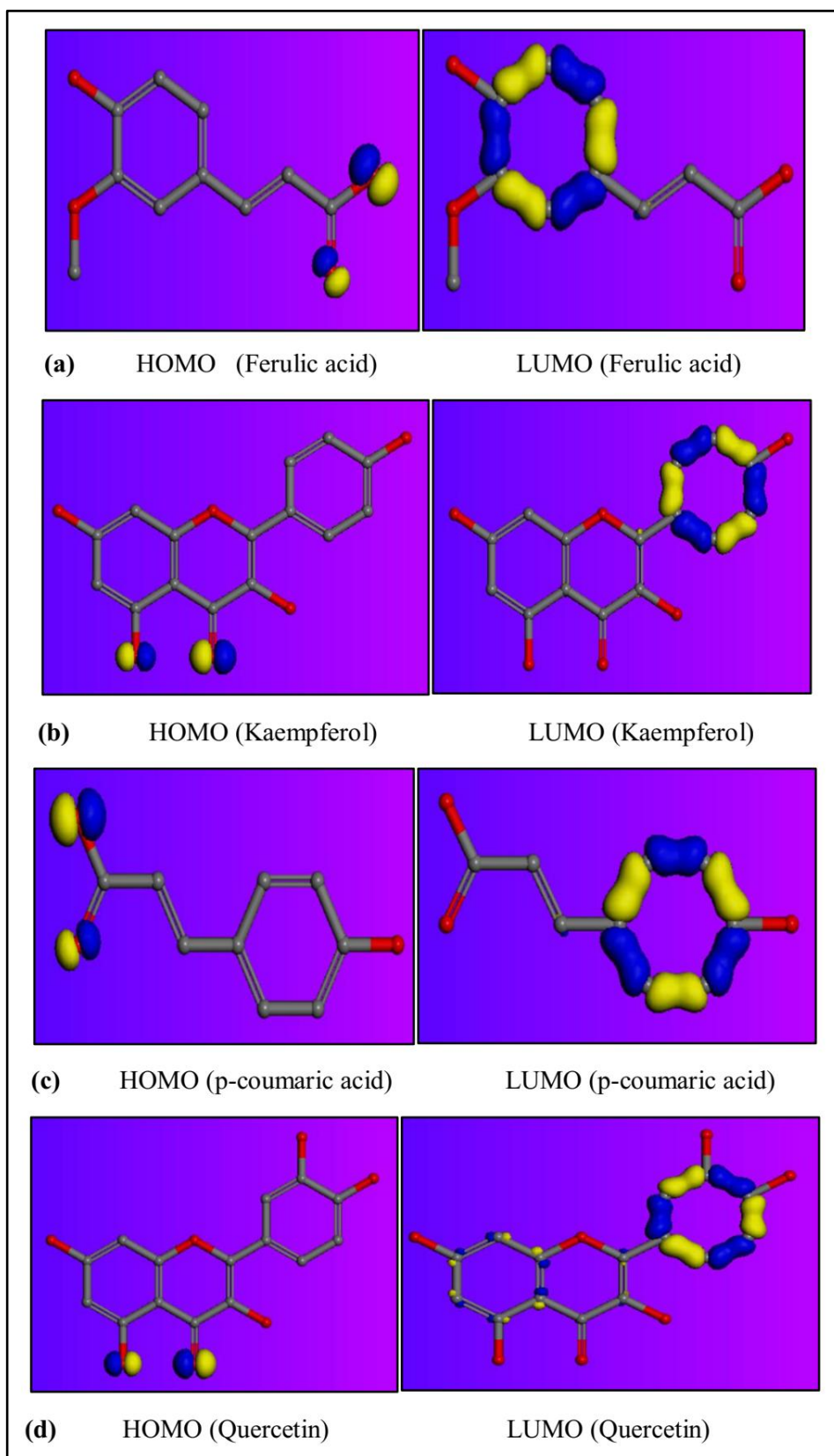
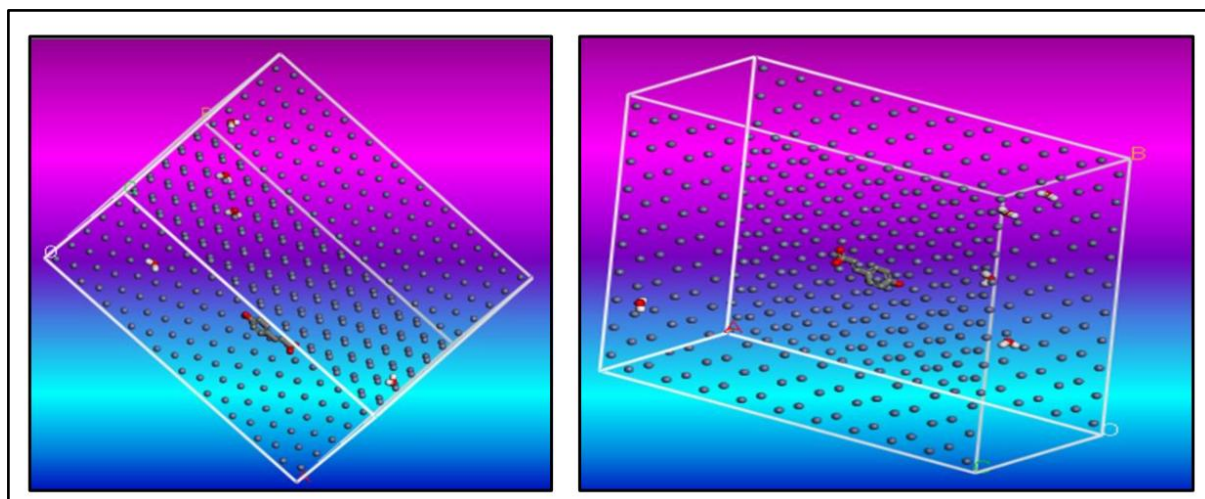


Fig. 13 Frontier molecular orbital (FMO) distributions of the major phytochemical constituents identified in *Allamanda cathartica* leaf extract used for DFT analysis: (a) ferulic acid, (b) *p*-coumaric acid, (c) kaempferol, and (d) quercetin, illustrating HOMO and LUMO electron density localization.



(a)

(b)

Fig. 14 Optimized adsorption configurations obtained from Monte Carlo simulations showing interactions between selected phytochemical ligands and the ZnO (110) surface under aqueous conditions: (a) side view and (b) top view of the adsorption geometry.

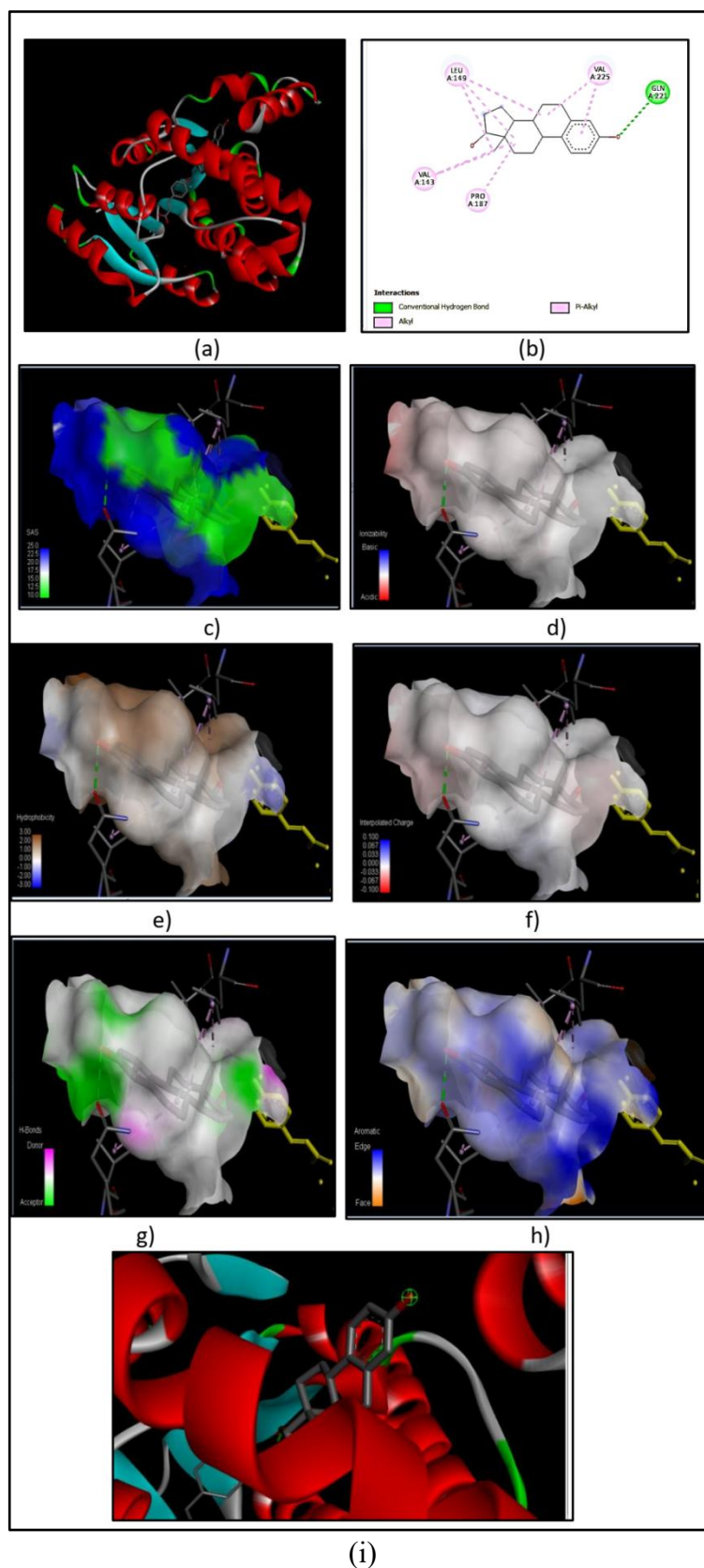


Fig. 15 Two-dimensional interaction diagrams of docked phytochemical ligands with human 17 β -hydroxysteroid dehydrogenase type 1 mutant (PDB ID: 1FDW) obtained from molecular docking analysis: (a–i) showing hydrogen bonding, hydrophobic interactions, and electrostatic contacts within the binding pocket.